Weight Cycling - Induced Alteration in Fatty Acid Metabolism

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A thesis submitted in partial fulfillment of the requirement for the degree of Master of Philosophy
in
Biochemistry

June 1998
Department of Biochemistry
The Chinese University of Hong Kong

Acknowledgments

I would like to express my sincere gratitude to my supervisors, Dr. Z. Y. Chen and Dr. W. P. Fong for their guidance and advice during the entire period of my study.

I would also like to express my appreciation to Dr. W. T. Cheung for his guidance and valuable advice in the study of adipocytes.

I am grateful to Mr. P. T. Chan and Mr. K. Y. Kwan for their assistance in many of the animal experiments. Thanks are due to Miss H. Y. Mok for her assistance during last summer. I also wish to thank my colleagues working in Room 131 for their help and technical support.

I must also express my deepest thanks to my parents, my brother, Mr. H. Y. Chan and his family. Their understanding and encouragement have enabled me to endure the hard times in my study.

Abstract

The present study was to examine the effect of weight cycling on lipid metabolism. By using Sprague Dawley rats as an animal model, two consecutive weight cycles were induced by 100%, 60% and 36% food restriction on chow diet (10% total energy from fat) in three separate experiments. The results of all the three experiments were similar. There was a selective reduction of linoleic and α-linolenic acids in carcass and adipose tissue lipids in weight cycled rats. Two weight cycles also remodeled the composition of adipose tissue triacylglycerols with an accumulation of saturated triacylglycerols species and a depletion of linoleate-enriched species. These changes occurred most likely as a result of preferential oxidation of linoleic and α-linolenic acids during the energy restriction, and subsequent biosynthesis of saturated and monounsaturated fatty acids. Thus, less linoleate-enriched triacylglycerol species and more palmitate-enriched triacylglycerol species were synthesized in adipose tissue during the *ad libitum* refeeding.

The effects of high-fat (45% energy) and medium-fat (22% energy) diets on weight cycling were also examined. Interestingly, the two weight cycles caused the weight-cycled rats fed high-fat diet to have a considerable increase in the weight of epididymal and perirenal fat pads. The size of adipocytes were also significantly enlarged by 80% compared with that in the weight-cycled rats than their corresponding controls. Furthermore, regardless of the rats fed on high-fat or medium-fat diet, all weight-cycled rats showed a gradual reduction of linoleic and α-linolenic acids but an increase of saturated fatty acids in adipose tissue and carcass total lipids.

It was found that these alterations of fatty acid composition occurred solely in carcass triacyglycerols but not in carcass phospholipids or carcass free fatty acids.

In addition, the influences of weight cycling on adipose and hepatic lipogenesis were studied by determining the activities of lipogenic enzymes and lipoprotein lipase. An overshoot of the activities of these enzymes was found in both weight-cycled rats fed on high-fat or medium-fat diet during the refeeding. Moreover, the overshoot was more pronounced in weight-cycled rats fed high-fat diet. These results confirmed the findings of the alteration in fatty acid composition and the hypothesis that "weight cycling with a high-fat diet may induce obesity".

Overall, the present study indicates that individual fatty acids respond differently to the weight cycling with a proportional depletion of linoleic and α-linolenic acid reserves and the subsequent changes in balance among polyunsaturated, monounsaturated and saturated fatty acids. These changes in tissue fatty acids are determined by the number of the weight cycling, the level of dietary fat, the differential β-oxidation of fatty acids, the activation of lipogenic enzymes and the lipoprotein lipase.

體重波動對脂肪代謝的影響

摘要

本論文主要是研究體重波動對脂肪代謝的影響。根據限制進食程度的不同，將實驗分爲 100%,60%和 36%節食三部份。鼠食爲一般常用的鼠食，脂肪含量爲總能量的 10%。實驗結果表明，儘管節食程度不同，所有體重波動鼠都出現同樣脂肪酸代謝的變化。透過兩個節食進食循環引起的體重波動，老鼠體內亞油酸和亞麻酸含量降低。另外，實驗結果表明，體重波動鼠體內含有較高的飽和脂肪酸的甘油三脂，而多不飽和脂肪酸的甘油三脂含量則較少。這些改變可能是因爲在禁食飢餓的刺激下，脂肪釋放亞油酸和亞麻酸增多；與此同時，這些脂肪酸的 β 氧化作用增強。另一方面，節食期後的隨意進食可刺激肝臟和脂肪組織飽和脂肪酸的合成。結果導致有較少的亞油酸和亞麻酸的甘油三脂，卻有較高的飽和脂肪酸的甘油三脂。

第二個實驗的設計是研究高脂肪(45%能量)和中脂肪(22%能量)的膳食對體重波動鼠的影響。實驗結果顯示，進食高脂肪食料的波動鼠的脂肪組織重量有顯著地增加($p<0.01$)。另外，它們的脂肪細胞體積比對照鼠的脂肪細胞增大了約 80%。而在脂肪酸成份方面，不論是進食高脂肪或中脂肪鼠食，體重波動鼠體內的亞油酸和亞麻酸的含量也降低，而飽和脂肪酸如棕櫚酸的含量則增加。

本論文的最後部份主要是觀察體重波動期間肝臟和脂肪組織的脂肪酸合成的變化。結果表明，在節食期後的隨意進食時段中，體重波動鼠的多種肝臟和脂肪

組織合成酶和脂肪組織脂蛋白脂酶的活性有明顯的增加。而這些增加在進食高脂肪鼠食的體重波動鼠中更爲顯著。這個發現印証了我們先前實驗的結果。

總括來說，此研究說明，節食引起的體重波動會引起老鼠體內亞油酸和亞麻酸含量降低及改變體內飽和脂肪酸和多不飽和脂肪酸之間的平衡，而這個改變都會受著體重波動的數目、脂肪酸的 β-氧化作用、以及脂肪酸合成酶和脂蛋白脂酶活化的影響。

List of Abbreviations

AA	Arachidonic acid
ACC	Acetyl-CoA carboxylase
BAT	Brown adipose tissue
CHD	Coronary heart disease
CTL	Control
DHA	Docosahexanoic acid
EPA	Eicosapentadecanoic acid
FAS	Fatty acid synthase
FFA	Free fatty acids
GLC	Gas liquid chromatography
HF	High-fat
HSL	Hormone sensitive lipase
LA/L	Linoleic acid/Linoleate
LDL	Low density lipoprotein
α-LnA/Ln	α-Linolenic acid/Linolenate
LPL	Lipoprotein lipase
LTs	Leukotrienes
M	Myristate
ME	Malic enzyme
MF	Medium-fat
MUFAs	Monounsaturated fatty acids
NIH	National Institutes of Health
O	Oleate
P	Palmitate
PEPCK	Phosphoenolpyruvate carboxykinase
PGs	Prostaglandins
PK	Pyruvate kinase
PL	Phospholipids
Po	Palmitoleate
PUFAs	Polyunsaturated fatty acids
S	Stearate
SD	Standard deviation
SDH	Succinate dehydrogenase
SFAs	Saturated fatty acids
TG	Triacylglycerol/Triglycerides
TL	Total lipids
TLC	Thin layer chromatography
TO	Trioleoylglycerol
TXs	Thromboxanes
UCP	Uncoupling protein
VLCD	Very low calorie diet
VLDL	Very low density lipoprotein
WC	Weight cycling/Weight cycle/Weight cycled

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Chapter 1

General Introduction

Weight cycling (WC) is a prevalent feature of the battle waged by both males and females, particularly among overweight individuals, against their excessive fatness. It is also a common practice of bulimic individuals.

Weight cycling was not perceived as an important issue until Kelly Brownell and colleagues called an attention to this phenomenon (Brownell *et al.*, 1986). They suggested that losing and regaining body weight made subsequent weight loss more difficult. Since then a new field of experimental and clinical research has emerged and weight-cycling has been under intensive investigation at several institutions in the United States and other countries (Anonymous, 1989). Recently, the Diet and Health Report of the National Academy of Sciences USA notes the possible detrimental effects of weight-cycling (National Academy of Science, 1989). Meanwhile, the Surgeon General's Report on Nutrition and Health recommends that the health consequences of weight cycling should be treated as "special priority" (Brownell and Rodin, 1994). Weight cycling has also gained wide attentions in both scientific field and public press.

1.1 Definition

Weight-cycling is the repeated loss and regain of body weight. It is often due to dieting. Since the dieting habit leads the body weight to a pattern of ups and downs like a "yo-yo" , hence it is also called "yo-yo" dieting (Figure1.1).

Neither the magnitude nor the number of the weight changes is well defined. A weight cycle can range from small weight losses and gains (5-10 lb. per cycle) to large changes in weight (50 lb. or more per cycle).

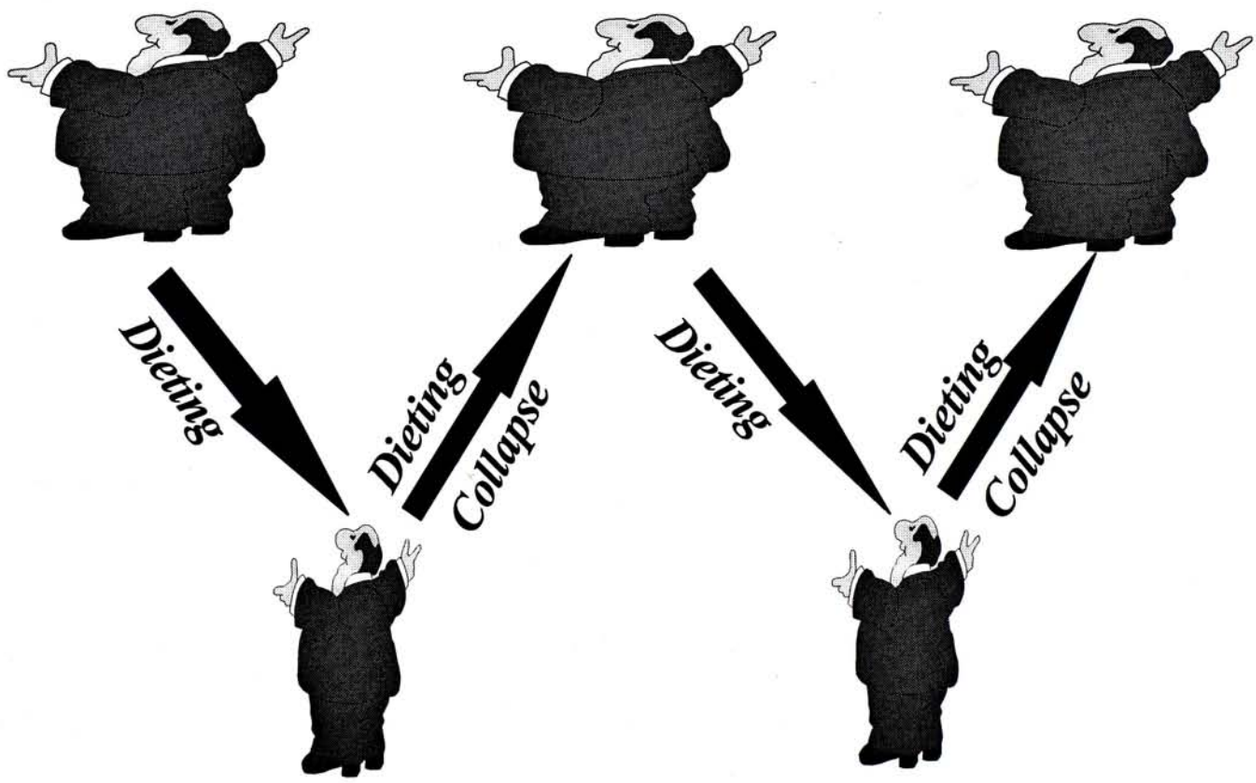


Figure1.1 Weight-Cycling as a result of "yo-yo" dieting.

1.2 Motivation of the Onset of Weight Cycling

There is little doubt that weight-cycling is prevalent in the world. About 60 to 80 million people are trying to lose weight and most of those who lose weight apparently regain it fairly quickly (Berg, 1994; 1995). The reasons for people to develop weight-cycling may vary, including stress, illness and obesity.

In the affluent societies, being overweight or obese becomes very common and it has reached epidemic proportions in the North America and Europe. Thus, obesity or overweight is one of the prime contributors to chronic disease development and results in a high mortality rate (Berdanier, 1991; Rolls, 1992). According to the report of Consensus Development Conference on the Health Implications of Obesity held by the National Institutes of Health (NIH) in 1985, a body weight of 20 percent or more above the desirable body weight constitutes a health hazard. In general, mortality increases with an increase in relative body weight. An obese individual is associated with an increased risk for hypertension, high blood cholesterol level, diabetes and coronary heart disease (CHD). Moreover, obese people are at higher risk in developing cancer. Thus, the major issue relative to the effect of the degree of general overweight in the population requires closer study.

On the other hand, from the present point of view, the most important criterion of being charming, beautiful or attractive is slim or thin. On the contrary, being fat is ridiculed, teased and excluded. Thin people, in other words, are walking paradoxes--having, yet metaphorically disowning, what everybody wants and envies. Furthermore, the press tries hard to promote this belief (Visser, 1993). Therefore,

most of the people including those who are actually not overweight attempt to slim down in order to improve their body images.

Among these people, more than half chose dieting as a means to lose their body weight. It is because they believe that dieting is the cheapest, easiest, most direct and most effective way to reduce their body weight. However, evidence shows that 80 to 90 percentage of the attempts doesn't work (Foreyt and Goodrick, 1993).

The psychological mechanisms involved in the maintenance of dieting behavior are particularly insidious. They made overweight individuals believe that dieting would work if they had more willpower. In the first few days or weeks of dieting, mood may be elevated and energy expenditure increased. Weight is then lost. This gives the impression that dieting does work. Dieting takes its toll and the dieters become satisfied and lose their impulse. Soon, the caloric restriction leads the dieters to uncontrollable cravings, especially for the high-fat foods. Relapses occur and are often characterized by reports of inability to control behavior or to think logically about food. In adverse cases, dieters may develop a binge eating pattern which is followed by periods of increased dietary restraint or even fasting (Figure 1.2). This dieting habit is repeatedly performed. As a result, weight cycle occurs due to "yo-yo" dieting (Deurenberg and Hautvast, 1989; Ferguson and Spitzer, 1995; Carmody *et al.*, 1995).

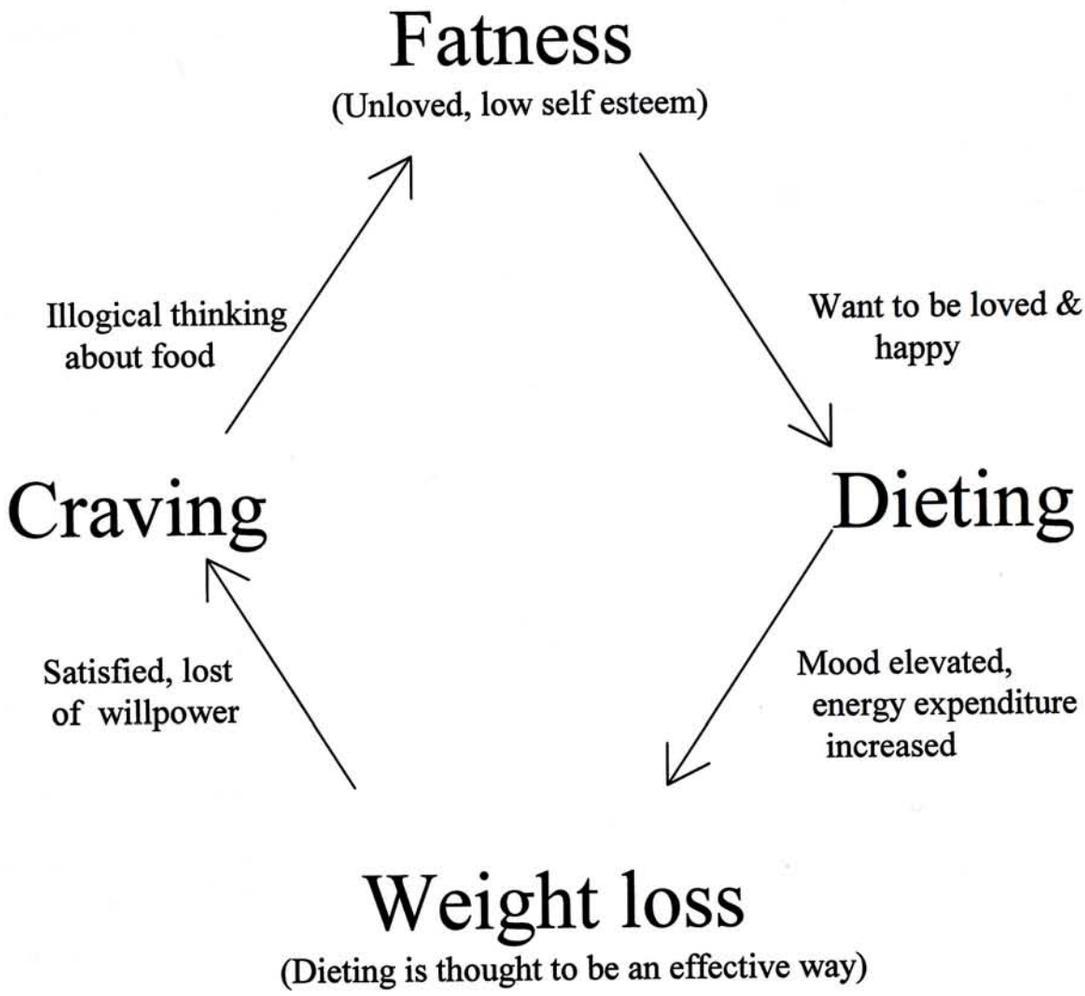


Figure 1.2 Psychological mechanism of dieting.

1.3 Physiological Effects of Weight Cycling

Recent studies involving both animal and human subjects have explored the hypothesis that weight cycling or the fluctuations in body weight over time have adverse effects on health (Lissner, 1989; Robert and Williams, 1989; Wannamewthree and Shaper, 1990a). Weight cycling has been shown to result in a "dieting-induced obesity" in rats (Bjorntorp and Yang, 1982; Bjorntorp *et al.,* 1982) and to be associated with a higher risk of coronary heart disease and premature death in humans (Hamm *et al.,* 1989; Wannamwthree and Shaper, 1990b; Lee and Paffenbarger, 1992).

1.3.1 "Dieting-Induced Obesity" Hypothesis

The hypothesis of "dieting-induced obesity" remains controversial. This hypothesis implies that repeated dieting inhibits weight loss but promotes weight regain. That is, one gets fatter because of dieting. This is not due to the psychological problems but is due to the physiological adaptation of dieting which causes metabolic alteration in energy balance. It is characterized by increased food efficiency or metabolic efficiency to prevent loss of body fat.

1.3.1.1 Food Efficiency

Studies have shown that repeated cycles of weight loss and regain in rodents and humans (Reed *et al.,* 1988; Steen *et al.,* 1988; Desautels and Dulos, 1988; Archambault *et al.,* 1989) caused an increase in food efficiency. That is, the caloric requirements for maintenance per gram of body weight were substantially reduced by previous dieting experience. In other words, small energy intake can give a high

energy accumulation and can be expressed mathematically as follows (Brownell *et al.*, 1986):

$$\text{Food Efficiency} = \frac{\text{Total energy accumulated (kcal)}}{\text{Total energy intake (kcal)}}$$

In the study of Brownell *et al.* (1986) , weight-cycled rats were more food efficient than the control rats after the second weight cycle. In his experiment, there were three groups of adult male Sprague-Dawley rats :

i) Chow Controls (CC) -------a normal weight control group, *ad libitum* access to chow throughout;

ii) Obese Controls (OC) -------obese animals, *ad libitum* access to a high fat diet throughout;

iii) Obese Cycling (OWC) ----obese animals, cycled through two bouts of caloric restriction (with 50% of the average intake of the chow consumed by the chow control) and refeeding (the rats resumed *ad libitum* access to the high fat diet).

The body weights of the rats as well as the food intake were measured daily except on weekends. In the first cycle, OWC animals required 21 days to loss weight to attain the weight of the CC (mean weight loss is 131g). In the second cycle, they required 46 days to loss weight equated that of the first restriction (mean weight loss is 133g). For the refeeding phases, 46 days were required for the OWC animals to regain to the weight of the OC in the first refeeding (mean weight gain is 131g) but only 14 days were required to regain the same weight in the second refeeding (mean weight gain is 131g and $p<0.0001$) (Figure1.3).

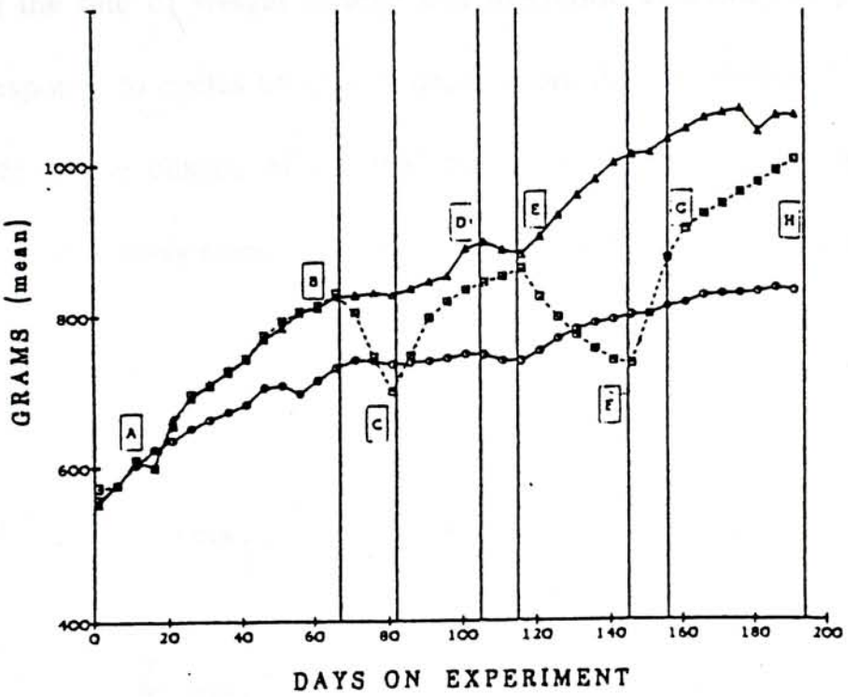


Figure1.3 Body weight changes in CC (●), OC (▲), and OWC (■), at the beginning of the experiment (A), beginning of the first restriction (B), end of the first weight loss period (C), point of weight regain to pre-diet weight (D), initiation of the second diet cycle(E), end of the second weight loss period (F), point of weight regain to pre-diet weight (G), and end of experiment (H) (Brownell *et al.*, 1986).

Although the daily food intake was the same during both restrictions, weight loss was less rapid in OWC. That means, food efficiency of OWC was significantly greater in the second cycle. In the first cycle, food efficiency is 0.0411 and is 0.0627

in the second cycle (Figure 1.4). Moreover, when compared with the OC group which got a food efficiency equal to 0.0052 at the end of the experiment, there is a four-fold increase in food efficiency (0.022). Therefore, this experiment demonstrated that weight cycling makes future attempts of weight loss even more difficult. These changes in the rate of weight change and metabolic efficiency is proposed as an adaptive response to cycles of energy deprivation. An repeated deprivation of food would increase the chance of survival by conserving energy during scarcity and converting food to body stores more efficiently when food is available again.

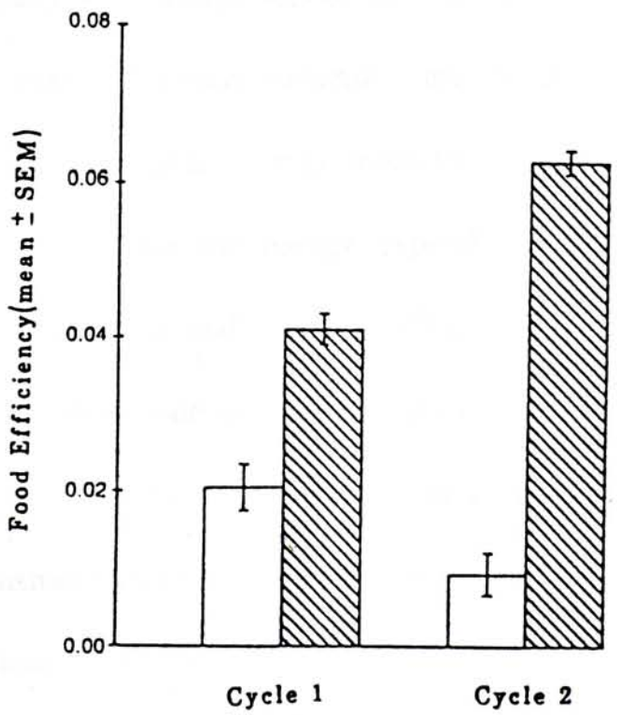


Figure 1.4 Food efficiency for OC and OWC during the two regain periods. Cycle 1 and cycle 2 refer to the periods C-D and F-G, respectively, in Figure 1.3. Open bars are OC and crosshatched bars are OWC (Brownell *et al.*, 1986).

1.3.1.2 Proposed Mechanisms for the Increase of Food Efficiency

It is believed that the weight-cycled animals increase their food efficiency or metabolic efficiency in order to increase their energy conservation. The mechanisms by which the weight-cycled animal achieve this higher food efficiency may include reductions in both resting metabolic rate and non-shivering thermogenesis. In addition, they may also increase the influx of energy substrate from plasma to peripheral tissue together with an increased activity of intestinal enzymes in order to absorb more energy-yielding nutrients.

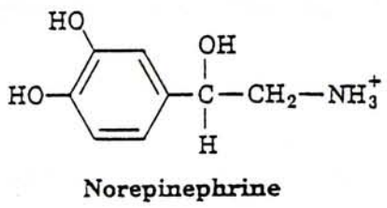
a) Reductions in resting metabolic rate and non-shivering thermogenesis

Recent researches with high school wrestlers give evidence that weight-cycled individuals may have a lowered metabolic rate (Steen *et al.,* 1988; Berg, 1989). Experiments showed that under energy restriction, the basal metabolic rate will be reduced in order to reduce the energy expenditure (Graham and Searle, 1975; Westerterp, 1977; Williams and Senior, 1979). Keys *et al.* (1953) showed that a depressed basal metabolic rate in adult humans induced by food restriction was still 30% below normal 8 weeks after commencement of rehabilitation. Similarly, Boyle *et al.* (1981) demonstrated that rats reduced their resting metabolic rate during restricted feeding and realimenation by the observation of reduction of oxygen consumption and locomotive activity. Reduction in resting metabolic rate lowers energy expenditure and increases energy efficiency. Thus, a greater percentage of dietary calories is partitioned into fat synthesis upon refeeding.

It is well documented that energy expenditure reduction appears in cycling mice as shown by a reduction of norepinephrine-stimulated oxygen consumption. This is probably due to a noticeable reduction in brown adipose tissue (BAT) thermogenic capacity. Non-shivering thermogenesis is a process of body heat generation through substrate-cycling in BAT, which is a "biological heating pad" with numerous mitochondria for uncoupling of oxidative phosphorylation and it is under the regulation of norepinephrine (Figure1.5).

Under stimulation of norepinephrine, adenylate cyclase of the norepinephrine receptor system synthesizes cAMP, which, in turn, allosterically activates cAMP-dependent protein kinase and then activates hormone-sensitive triacyglycerol lipase by phosphorylation. Finally, the activated lipase hydrolyzes triacyglycerols to release the free fatty acids that open the proton channel formed by the uncoupling protein (UCP) which is a protein translocator and induces the uncoupling of oxidative phosphorylation (Voet and Voet, 1995).

It was shown that repeated fasting-refeeding (1 day of fasting followed by 2 days of refeeding) caused marked atrophy of BAT with a significant reduction of BAT succinate dehydrogenase (SDH) content and a reduction in the mitochondrial concentration of UCP (Boyle *et al.,* 1981; Desautels and Dulos, 1988). As both SDH and UCP are important for oxidative phosphorylation, the reduction in these two proteins will decrease the metabolic activity and suppress the norepinephrine-induced thermogenesis. Therefore, it is suggested that weight-cycling will increase the energy conservation by reducing energy expenditure.



Norepinephrine

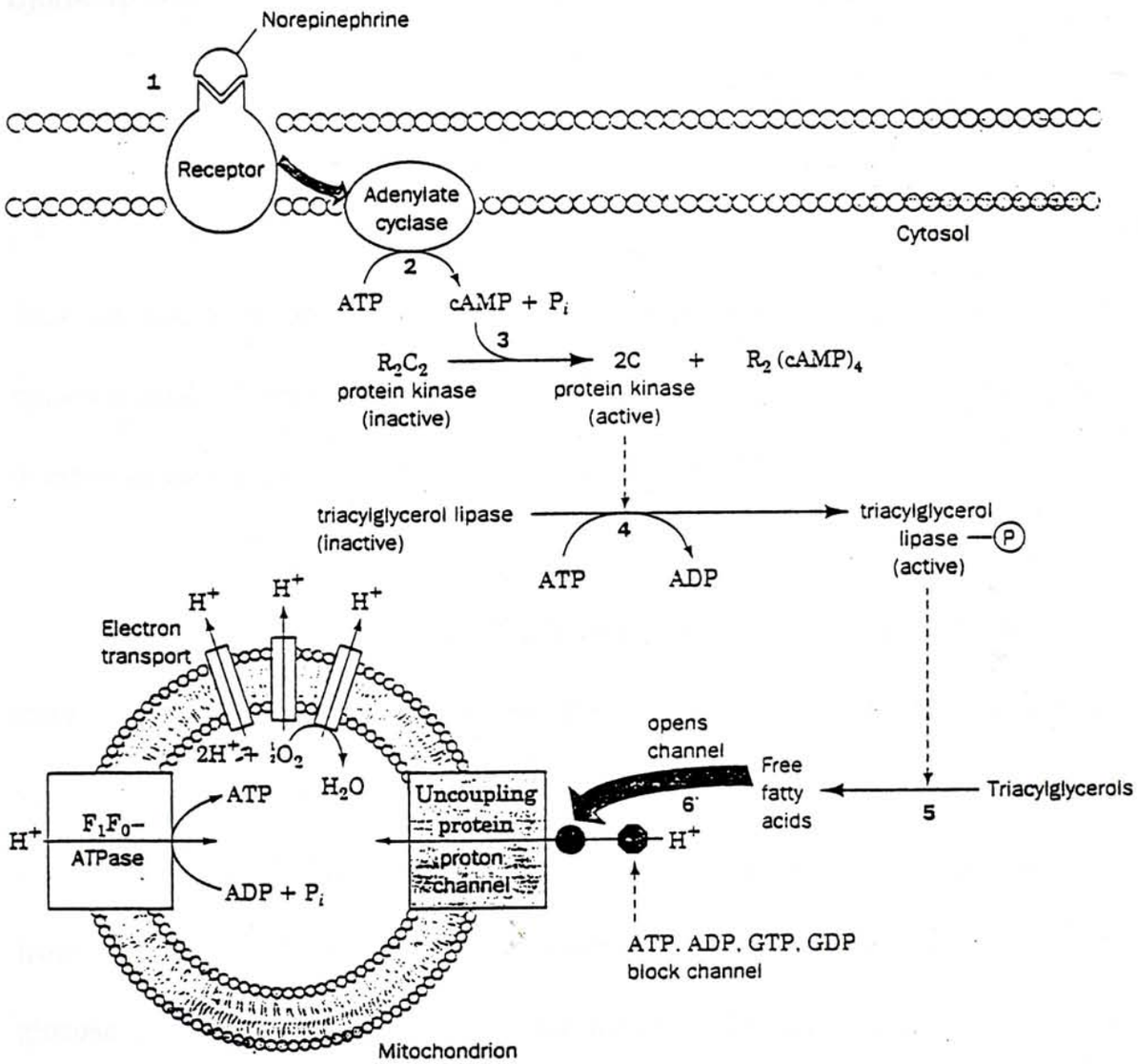


Figure1.5 The mechanism of hormonally induced uncoupling of phosphorylation in brown fat mitochondria (Voet and Voet, 1995).

b) Energy substrate influxes from plasma to peripheral tissue

Studies in fasted-refed rats showed reductions in blood glucose with concomitant increased metabolism of glucose by peripheral tissues. This suggests that a "pull' of substrate into the peripheral tissue compartments induced by the dieting experience would affect the caloric intake after dieting (Bjorntorp *et al.,* 1980; Bjorntorp *et al.,* 1982).

Bjorntorp *et al.* (1982) demonstrated that plasma glucose and triglyceride were lower in the weight-cycled rats. Moreover, their glucose turnover rate was increased. That implied both gluconeogenesis and glucose uptake were elevated in weight-cycled animals. Furthermore, it was also found that the weight-cycled rats showed an overeating during the first few days of the refeeding period.

These results further support the hypothesis that depletion of energy substrate stores in fasting rats is followed by an efficient repletion of energy to their previous sizes during *ad libitum* refeeding. When refeeding starts after fasting, refilling of energy stores is probably occurring with an increased flux of glucose and triglycerides from circulation to the adipose tissues as indicated by a lower plasma concentration of glucose and triglycerides. The increased substrate flux and the efficient capture of energy substrate by the peripheral tissue give a marked increase in the efficiency for accumulating energy. Also, the experience of fasting stimulates the brain to signal the body to consume more food with a preference for dietary fat (Reed *et al.,* 1988; Contreras and King, 1989). It seems likely that these adaptations are significant for the inability of obese subjects to maintain a lower body weight after weight reduction.

c) Change of Intestinal Enzymes

A possible explanation for the adaptation of peripheral tissues to efficiently capture the energy substrate is that the enzyme activity is increased in various anabolic energy pathways. The experimental evidence did show that the specific activities of intestinal enzymes rose to supernormal levels during refeeding of the fasted-refed rats (Kotler, 1982). These enzymes included surcrase, maltase, hexokinase, and pyruvate kinase. Moreover, the specific activity of thymidine kinase did not fall with fasting but rose above the control levels during refeeding. The similar response to refeeding by thymidine kinase as well as the disaccharidases and glycolytic enzymes implies that the adaptive response to refeeding in enzymes activities may be also occurring elsewhere in the body. These changes may explain the increased rate of disappearance of plasma glucose and the increased rates of glycogen and triglyceride synthesis noted in refed animals (Bjorntorp *et al.,* 1982).

The precise mechanism by which enzyme activities increased above the control values is unclear but it is known that the intestinal enzyme activities are affected by specific luminal, neural and hormonal signals. Thus, the weight cycling treatment may give some signals to the brain which, in turn, co-ordinates the body to have a series of adaptations including an increase in intestinal enzyme activities in order to increase the capture of energy.

1.3.1.3 Change in Body Fat

When adult rats are refed *ad libitium* after a short period of starvation, they rapidly regain all lost weight, and body weight soon equals that of the controls

(Hollenberg and Vost, 1968; Hirsch and Han, 1969; Levitsky *et al.,* 1976). Although it was found that the weights of carcasses between the weight-cycled rats and controls are similar, their body composition might be different. It is because water accounts for more than half of the losses during fasting but during *ad libitium* refeeding, weight regains as both protein and more fat together with water (Szepesi, 1975; Szepesi and Vojnik, 1975; Szpesi *et al.,* 1977; William, 1979; Bjorntorp and Yang, 1982). Therefore, it has been hypothesized that after successive weight cycles, more and more body fat is deposited. A recent study by Ernsberger *et al.* (1996) demonstrated that 3 weight cycles of very low calorie diet (VLCD; 16.7% of baseline calories) followed by refeeding increased the accumulation of adipose tissue in intra-abdominal depots.

1.3.2 Association with Increased Mortality and Coronary Heart Disease (CHD)

Several major epidemiological studies show that the increase in mortality from all causes and from coronary heart disease is associated with weight cycling (Hamm *et al.,* 1989; Lissner *et al.,* 1991; Lee and Panffenbarger, 1992; Ernsberger and Kolestsky, 1993; Brownell and Rodin, 1994). Among these studies, Hamm *et al.* (1989) were the first to present evidence suggesting that large fluctuations in body weight during young adulthood may increase risk for death due to coronary heart disease. Analyzing the data from the Western Electric Study in which 2107 men, age 40-55 years at baseline and followed up for over 25 years, they observed that the risk of death from CHD was twofold higher in men who gained and lost more than 10% body weight during any five-year period compared to men with less than 5% weight

change ($p<0.05$). The risk was adjusted for age, serum cholesterol, systolic blood pressure, cigarette smoking, alcohol intake and body mass index (weight in kilogram divided by the square of height in meters) reported at initial examination.

Furthermore, Lissner *et al.* (1991) utilized the long-term data from the Framingham Heart Study to assess the relation of body weight variability to total mortality, cancer morbidity, and CHD morbidity and mortality in 1804 women and 1367 men, age 30-62 years. The subjects were examined every two years for a total period for 32 years (Figure1.6). Measured changes in body weight in the first 14 years and the subjects-recalled weight at age 25 were used to categorize the direction and magnitude of any weight cycling. To control for the effect of pre-existing illness, the investigators waited for four years between the last measurement of weight and the first outcome counted at the ninth examination (Figure1.6). A multivariate analysis was used to exclude other known cardiovascular risk factors including smoking, serum cholesterol, systolic blood pressure, glucose tolerance, level of physical activity in addition to age, body mass index, and death from all causes. It was found that CHD still remained significantly associated with weight fluctuation both in men and women. Compared to that of the subjects with relatively stable body weight (1.00), the relative risk of mortality from coronary heart disease was 1.93 and 1.55 in weight cycled-men and weight cycled-women, respectively ($p<0.009$).

The underlying mechanisms contributing to this close relationship between weight cycling and mortality rate as well as CHD are unknown. However, weight cycling may account for the observed increase in deaths in at least several ways. One of these may be the increase of coronary heart disease risk such as hyperinsulinemia

and hypertension which have been shown to be the results of weight cycling in animals (Ernsberger and Nelson, 1988; Robert and Williams, 1989). Another possibility is that repeated weight loss and regain may promote fat deposition and central obesity (selective increase in abdominal adiposity) in both rodents and humans (Rodin *et al.,* 1990; Ernsberger *et al.,* 1996). As an abdominal pattern of fat deposition in humans has been shown to be associated with increase in cardiovascular risks, thus, increased abdominal obesity or central obesity is a potential avenue for deleterious effects. Furthermore, weight cycling may also interfere the lipid metabolism and disturb the balance of fatty acids which is favorable for CHD.

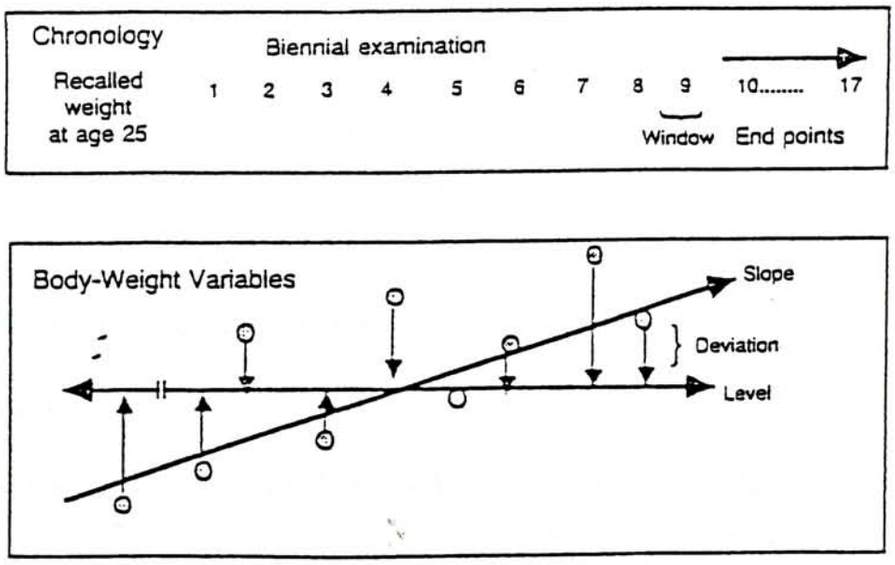


Figure1.6 Chronology and schematic of body-weight variables for a hypothetical subjects. The chronology illustrates the timing of measurements of body weight (made at intervals of two years, part of the Framingham Study) in relation to the occurrence of end points, with a four-year interval ("window") required between the measurement of weight at the eighth examination and the first end point included in the analysis. Weight was converted to body-mass index (BMI), defined as the weight in kilograms divided by the square of the height in meters, which was used to calculate three key independent variables: a subject's mean BMI (level), the linear trend in the BMI over time (slope), and the BMI's degree of variability from the mean (coefficient of variation). Determinations of the BMI are indicated by shaded circles and arrows (Lissner *et al.,* 1991).

Chapter 2

Depletion of Linoleic Acid and α-Linolenic Acid Caused by Weight Cycling is Independent of the Extent of Calorie-Restriction

2.1 Introduction

2.1.1 Nomenclature of Fatty Acids

Fatty acids are classified according to carbon chain length, location and number of double bonds. Physical and chemical properties are in part related to the carbon chain length of a given fatty acid, with 2-4 carbons being categorized as shorter-chain, 4-12 carbons being as medium chain, and 14-24 carbons being as longer chain. Fatty acids with no double bond are referred as saturated fatty acids (SFAs). Fatty acids with one double bond are called monounsaturated (MUFAs), and those having two or more double bonds are polyunsaturated fatty acids (PUFAs). PUFAs can be divided into four families or classes according to the location of the double bond closest to the methyl terminal of the carbon chain: n-3, n-6, n-7 and n-9. The structure of a given fatty acid can be briefly described by noting its overall carbon chain length, the number of double bonds, and the position of the first double bond. For example, linoleic acid (LA) has 18 carbon atoms in its carbon chain with two double bonds, the first being at the sixth carbon from the methyl terminal end, and is denoted as 18:2n-6. Table 2.1 shows the formulae and names for many of the common dietary fatty acids which are clinically and nutritionally important.

Table 2.1 Names and formulae of common fatty acids

Symbol	Common name
Saturated fatty acids	
10:0	Capric
12:0	Lauric
14:0	Myristic
16:0	Palmitic
18:0	Stearic
Monounsaturated fatty acids	
16:1n-7	Palmitoleic
18:1n-9	Oleic
Polyunsaturated fatty acids	
18:2n-6	Linoleic
18:3n-3	Linolenic
20:4n-6	Arachidonic
20:5n-3	Eicosapentanoic
22:6n-3	Docosahexanoic

2.1.2 Metabolism and Physiological Role of LA and α-LnA

Linoleic acid (LA) and α-linolenic acid (α-LnA) are considered as essential fatty acids as they are suggested to be synthesized *de novo* only by the chloroplasts in phytoplankton and in plants but not in mammals (Hassam *et al.*, 1979). However, animals are able to convert these parent fatty acids to longer-chain fatty acids by alterating desaturation and chain elongation, thus forming two distinct series of polyunsaturated fatty acids referred as n-6 and n-3 fatty acid classes (Figure 2.1).

Although LA and α-LnA are structurally different, they share or compete for the same enzymatic pathway to metabolize into corresponding longer chain PUFAs (Figure 2.1). The competition between n-3 and n-6 fatty acids for desaturases plays a critical role in determining which kind of fatty acid is preferentially metabolized. There is no inter-conversion between these two classes of fatty acid in animals since they are unable to desaturate the carbon chain of fatty acids with double bonds closer than carbon number 7 from methyl group. The conversion of parent fatty acids to their long chain derivatives is therefore especially important in the case of herbivores or vegetarians since little or no 20-carbon and longer chain PUFAs can be found in their diet.

LA and α-LnA have been recommended to be named as "indispensable" or "conditionally dispensable" fatty acids instead of "essential" fatty acids (Cunnane, 1996). It has been shown that the hexadecadienoate (16:2n-6) and hexadecatrienoate (16:3n-3) present in edible green plants can serve as precursors to synthesize LA and α-LnA respectively (Cunnane *et al.*, 1995). The absence of gross symptoms of long term LA and α-LnA deficiency in adults and humans is also one of the stand points of the argument (Aaes-Jorgensen, 1961). However, they play numerous vital physiological roles regardless of their essentiality.

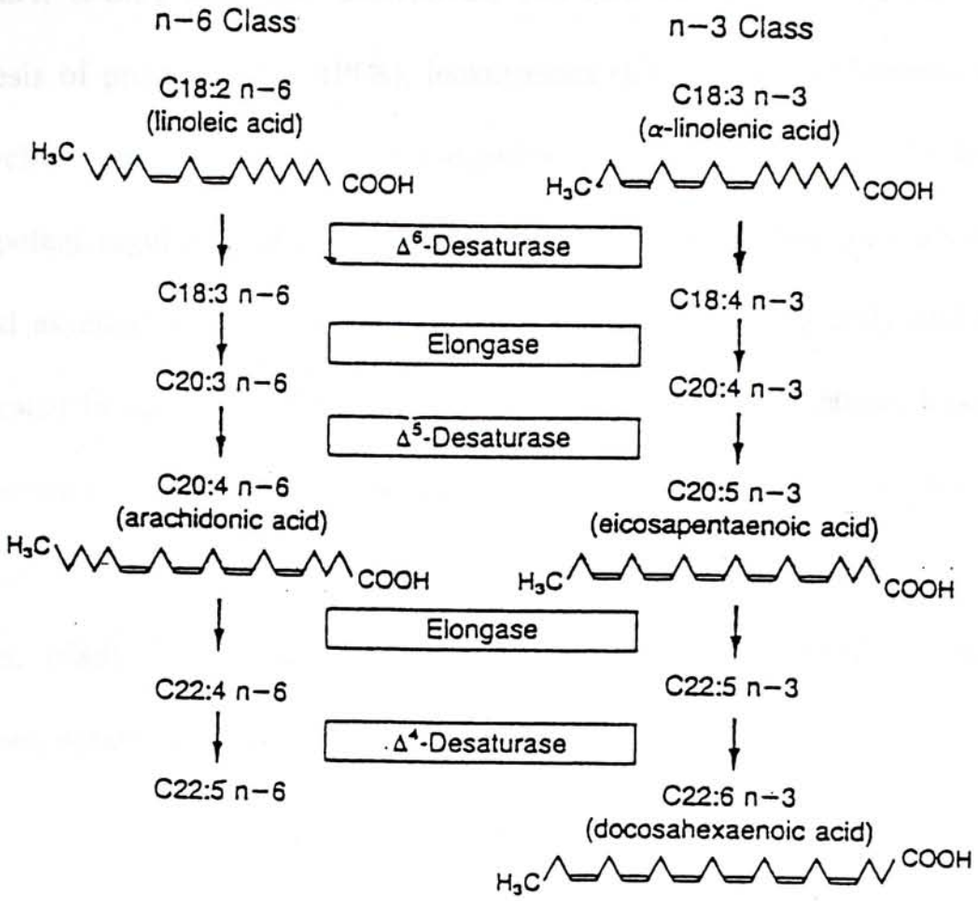


Figure 2.1 The principal PUFAs of the n-6 and n-3 series and their metabolic interconversions (Borkman *et al.*, 1993).

2.1.2.1 LA, α-LnA and their Derivatives as Structural Components

LA, and its derivatives, arachidonic acid (AA), eicosapentadecanoic acid (EPA) and the major n-3 PUFAs, docosahexanoic acid (DHA), are structural elements in cell membranes and other barriers in the body. They are especially abundant in brain phospholipids and are essential for the development and function of brain, retina, testes as well as sperm.

2.1.2.2 Production of Eicosanoids from LA and α-LnA

LA, α-LnA and their derivatives, AA and EPA, act as precursors for the synthesis of prostaglandins (PGs), leukotrienes (LTs), and thomboxanes (TXs), via the cyclo-oxygenase and lipoxygenase pathways (Figure 2.2). PGs, LTs and TXs are very potent regulators of immune function and differentiation and are collectively termed as eicosanoids. PGs are produced in all parts of the body and have been implicated in activities from the induction of labor to inflammation, blood pressure maintenance, and headaches (Bonaa *et al.,* 1990). LTs and TXs are alternative products involved in platelet aggregation and inflammatory response (Leaf and Weber, 1988). They have been presumably linked to the pathophysiology of some diseases, notably atherosclerosis.

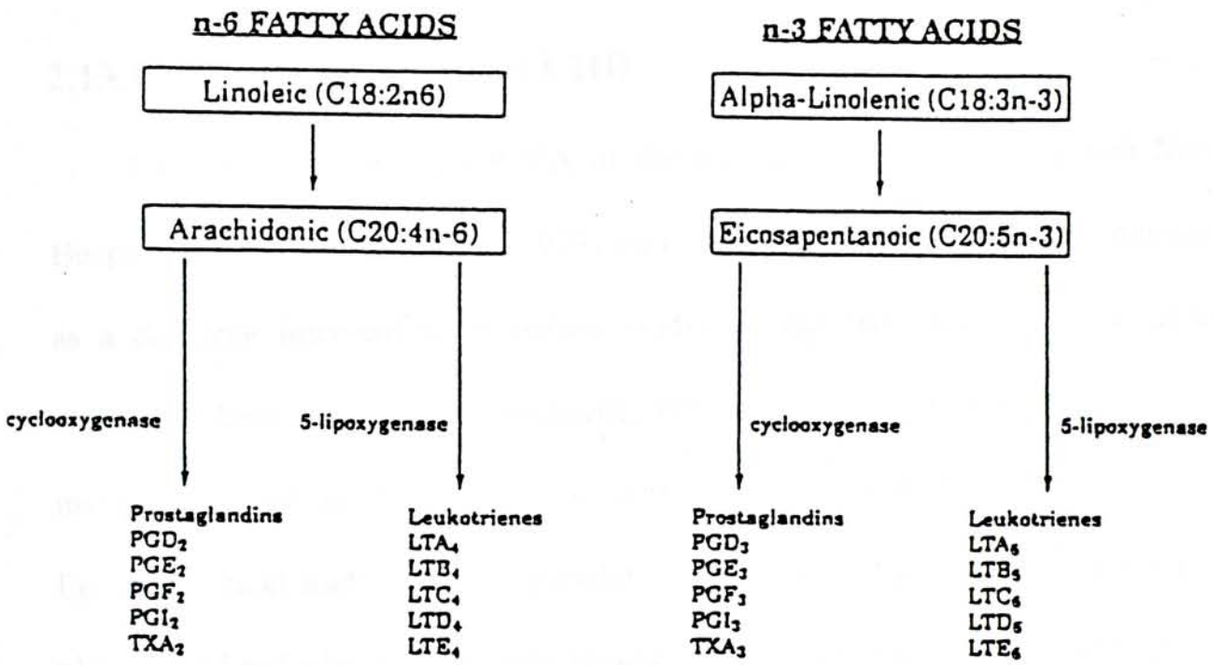


Figure 2.2 The metabolism of the AA and EPA to eicosanoids (Dyerberg, 1992).

2.1.2.3 Other Physiological Roles

LA and α-LnA are used in the *de novo* synthesis of non-essential fatty acids (Cunnane *et al.*, 1994). They are also needed in the esterification of plasma cholesterol, necessary for its uptake as low density lipoprotein (LDL) core material

and the normal excretion of sterols and bile acids (Mead, 1980). An additional newly found function of LA is that its derived lipid forms are needed to ensure the water impermeability of skin (Hansen and Jensen, 1985). It helps to explain the classic skin symptomology of essential fatty acid deficiency.

2.1.3 Dietary LA and α-LnA Relative to CHD

2.13.1 Dietary LA and CHD

LA is the principal n-6 PUFA of the diet. According to the Finnish Mental Hospital Study (Turpeinen *et al.*, 1979), an increase in dietary LA has been postulated as a desirable intervention to reduce cardiovascular risk. LA can lower plasma cholesterol levels (Nordy and Goodnight., 1982), while its content in adipose tissue is inversely related to blood pressure (Oster *et al.*, 1979; Singer *et al.*, 1982). Epidemiological studies in some populations have also suggested that a lower LA in plasma lipid and adipose tissue may be associated with an increased risk of CHD.

One of these reports is the Edinburgh-Stockholm Study (Logan *et al.*, 1978) which demonstrated that the mortality from CHD for males aged approximately 40 was three times greater in Edinburgh than that in Stockholm. At the same time, the relative LA content in plasma triglycerides, plasma cholesterol esters and adipose tissue triglycerides of Edinburgh men was significantly lower than that of Stockholm (Table 2.2; Figure 2.3).

Similar differences have also been observed in population selected randomly in Finnish region (Nikkari *et al.*, 1983) and Edinburgh region (Wood *et al.*, 1984). In

Finnish regional study, men from east Finland, where the mortality from CHD is almost twice as that in west Finland, have lower proportions of LA in serum lipids than their counterparts in west Finland. In Edinburgh regional study, it was found that those with hitherto occult CHD also had a significant lower proportion of LA in their adipose tissue. A crude inverse correlation can also be seen between plasma cholesterol linoleate level and mortality from CHD in Denmark, Sweden, the United States, Scotland and Finland (Oliver, 1981). This inverse relationship is further supported by another epidemiological study in Europe (Riemersma *et al.,* 1986).

Table 2.2 Linoleic acid in relation to CHD (Logan *et al.,* 1978)

Measurment	Edinburgh (107 men) mean ± (S.D.)	Stockholm (82 men) mean ± (S.D.)
% linoleic acid in		
Plasma cholesterol-esters	**48.4 (6.5)***	**56.4 (6.0)**
Plasma triglyerides	**10.8 (3.8)***	**18.2 (4.5)**
Adipose tissue	**7.3 (1.5)*****	**11.8 (2.1)**
Adipose tissue P/S Ratio	**0.30 (0.07)**	**0.44 (0.11)**

*p<0.05, ***p<0.001

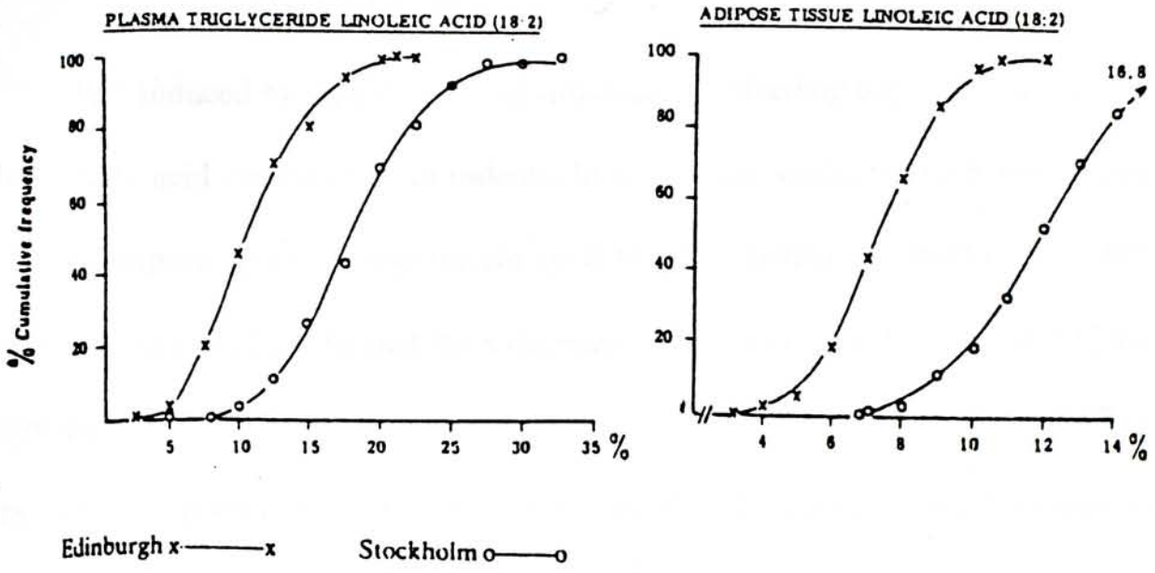


Figure 2.3 Linoleic acid content in Edinburgh men and Stockholm men (Logan *et al.*, 1978).

2.1.3.2 Dietary α-LnA and CHD

Regarding the effect of α-LnA on CHD, a study over a period of 4 years under similar conditions of 18 groups of male farmers from France, Great Britain and Belgium, comprising a total number of 460 subjects, identified a significant inverse relationship between the level of α-LnA in plasma lipids and mortality of CHD (Renaud *et al.*, 1986). α-LnA has been found to decrease the activity of platelet by the reduction of serum triglycerides and very low-density lipoprotein (VLDL) cholesterol, especially in subjects with hypertriglyceridemia, and at the same time, the elevation of high-density lipoprotein (HDL) cholesterol (Wood *et al.*, 1987; Seidelin *et al.*, 1992).

2.1.4 WC-Induced Alteration in the Composition of Tissue Lipids

WC induced by repeated fasting followed by refeeding appears to modify whole body fatty acid composition in rodents. In a previous study, the response of adipose tissue composition to a single weight cycle (48-h of fasting followed by refeeding) in pregnant rats is characterized by a decrease in LA and α-LnA (Chen and Cunnane, 1993b). Besides, Chen *et al.* (1996) have recently demonstrated that 4 WCs induced by 100% food restriction (24-h fasting followed by 72-h *ad libitum* refeeding) caused a significant quantitative decrease in LA and α-LnA but an increase in the accumulation of palmitate (16:0), palmitoleate (16:1n-7) and oleate (18;0) in carcass total lipids and in perirenal and epididymal adipose tissue of young growing rats (Figure 2.4). Despite an adequate n-6 and n-3 polyunsaturates has been provided in the diet during refeeding, weight cycling has still caused a moderate and selective depletion of LA and α-LnA from tissue stores.

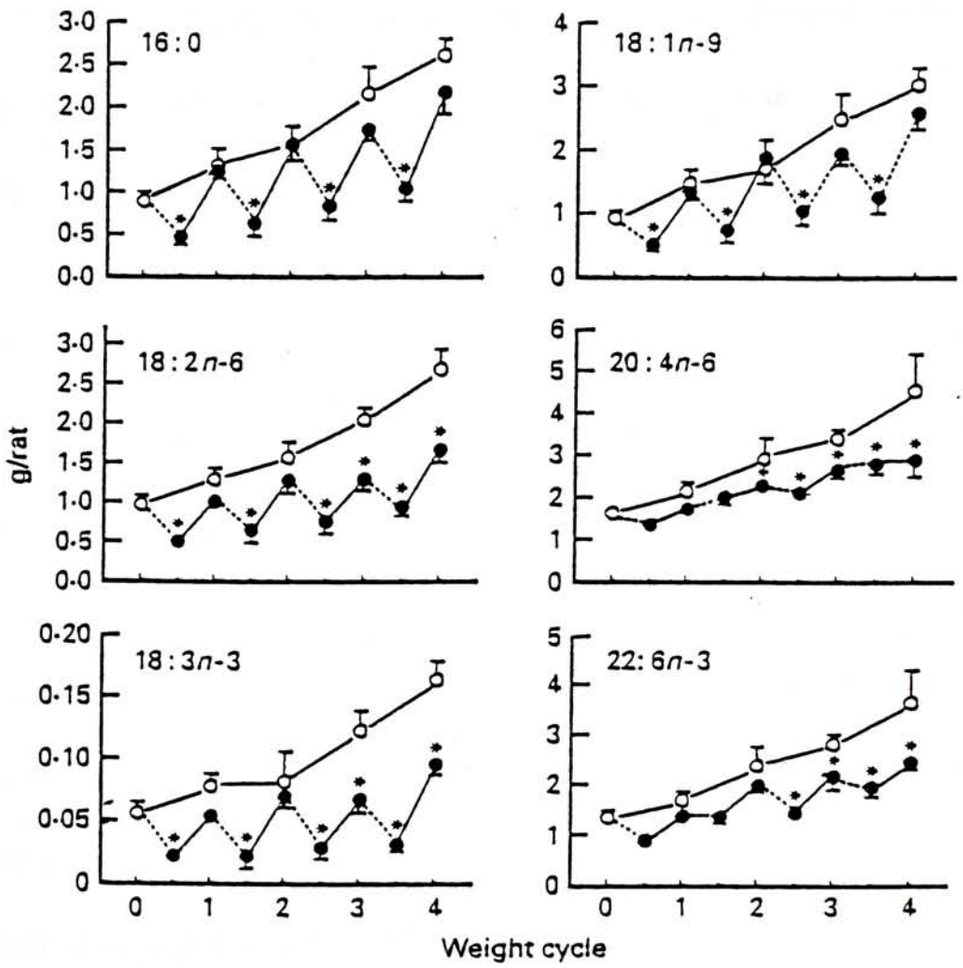


Figure 2.4 Changes in whole-body content of individual long-chain fatty acids during weight cycling in rats caused by 24 h fasting (●-----●) followed by 3 day refeeding (●——●) compared with free-feeding control (○——○). *Mean values were significantly different from those of control at $p<0.01$ (Chen *et al.*, 1996).

2.2 Objective of the Present Study

Although there may be a close relationship between WC and CHD, the underlying mechanism is unclear. It is reasonable to speculate that WC may change the lipid metabolism and favours CHD. The present study was therefore designed to investigate whether there are any alteration of lipid metabolism as a result of several WCs. These included the balance between body saturated fatty acids and polyunsaturated fatty acids, and quantitative changes in individual adipose tissue triacylglycerol species.

Previous studies showed that WCs prevented accumulation of LA and α-LnA, and led to depletion of these fatty acids from the body (Chen and Cunnane, 1993b; Chen *et al.,* 1995; Chen and Ratnayake, 1995). However, the WC induced by 100% calorie restriction (complete fasting) followed by *ad libitum* refeeding in those studies may not reflect the common practice in the weight-cycled individuals. Thus, in this study, different degree of food restrictions was applied in order to mimic the WC dieting pattern of humans. The objective was to test whether the selective depletion of LA and α-LnA associated with the WC was also true when the extent of food restriction used to induce weight reduction was different.

2.3 Materials and Methods

2.3.1 Animals and Diets

Forty-eight Sprague Dawley rats (335g) were housed with two rats per cage in an animal room at 23°C with 12h light/dark cycles. Their food intake and body weight were measured daily. The rats were fed a rodent chow diet (PMI, St. Louis, MO, USA) which contained 5 g fat/100 g diet. The fatty acid composition is shown in Table 2.3.

Table 2.3 Fatty acid Composition in the Chow Food Diet

Fatty Acid Composition	**wt% of Total Fatty Acids**
Palmitic acid (16:0)	20.5
Palmitoleic acid (16:1n-7)	2.4
Stearic acid (18:0)	8.7
Oleic acid (18:1n-9)	26.5
Linoleic acid (18:2n-6)	29.9
Linolenic acid (18:3n-3)	3.0
Arachidonic acid (20: 4n-6)	0.2
Eicosapentaenoic (20:5n-3)	1.1
Docosahexaenoic (22:6n-3)	0.9

The rats were stabilized on the diet for one week and their food intakes were 29-30 g diet/rat/day. They were then randomly divided into six groups. Three of them were the *ad libitum*-fed control (CTL) groups while the other three were weight-cycled (WC) groups. Each WC group was matched with its own CTL group. The CTL rats were allowed free access to the diet and tap water throughout the period of study while the WC rats were subjected to 2 fasting-refeeding cycles and only allowed free access to the food during the period of refeeding.

In experiment 1, the WC rats were completely fasted, i.e. 100% food restriction, for two days followed by *ad libitum* refeeding to reach the average weight of the CTL. In experiment 2, the two consecutive WCs were induced by 60% food restriction (12.5 g/rat/day) for a period of 4 days followed by *ad libitum* refeeding until the average body weight of the WC rats reached that of the CTL. In experiment 3, the two WCs were induced by 36% food restriction (18.5 g/rat/day) for a period of eight days followed by *ad libitum* refeeding. At the end of two WCs, all the rats were killed under nitrogen anaesthesia and exsanguinated via the abdominal aorta into a syringe. After clotting, the serum was separated from the whole blood by centrifugation (2000g for 15min). The liver, perirenal, and epididymal adipose tissue (one pad) were removed , washed with saline and frozen at -20°C. The lipids were extracted within one week. Carcass [whole body - perirenal adipose tissue (one pad) - epididymal adipose tissue (one pad) - liver (1 gram) - blood] was also retained for fatty acid analysis (Chen *et al.,* 1995). This study was approved by the Animal Care Committee of the Chinese University of Hong Kong.

2.3.2 Lipid Analysis

Total lipids of liver, adipose tissue and carcass were extracted using chloroform-methanol (2:1, vol/vol) containing 0.02% butylrated hydroxytoluene (Sigma Chemical, St. Louis, MO, USA) as an antioxidant. For the carcass samples, two aliquots of homogenized carcass were extracted as shown in Figure 2.5 (Chen *et al.*, 1996). Heptadecanoic acid (Sigma Chemical, St. Louis, MO, USA) was added as an internal standard (IS) to an aliquot of the total lipid extract to quantitate carcass total fatty acids. The lipid extracts containing the IS were converted to fatty acid methyl esters by using a mixture of 14% boron trifluoride in methanol (BF_3-MeOH) (Sigma Chemical, St. Louis, MO, USA) and toluene (2:1, vol/vol) under nitrogen at 90°C for 45 min (Figure 2.6). The fatty acid methyl esters were then extracted into 1 ml of hexane (Figure 2.7) and saved for the gas-liquid chomatographic analysis.

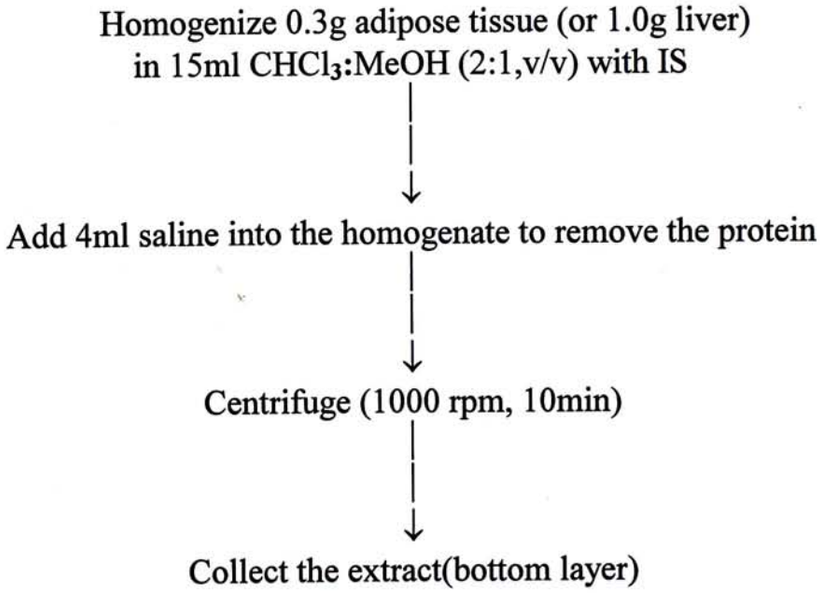


Figure 2.5 Flow chart of the extraction procedures.

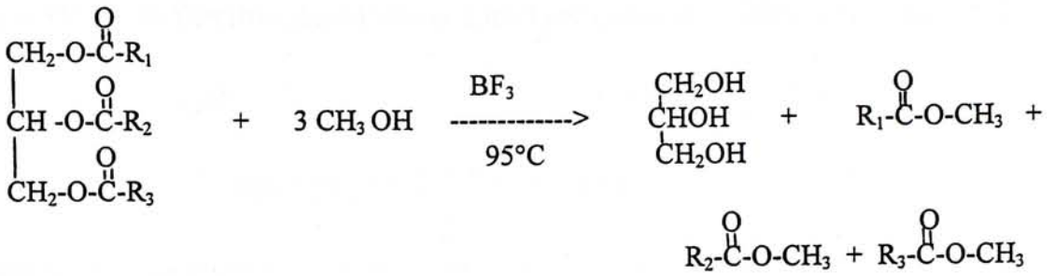


Figure 2.6 Chemical equation of the methylation.

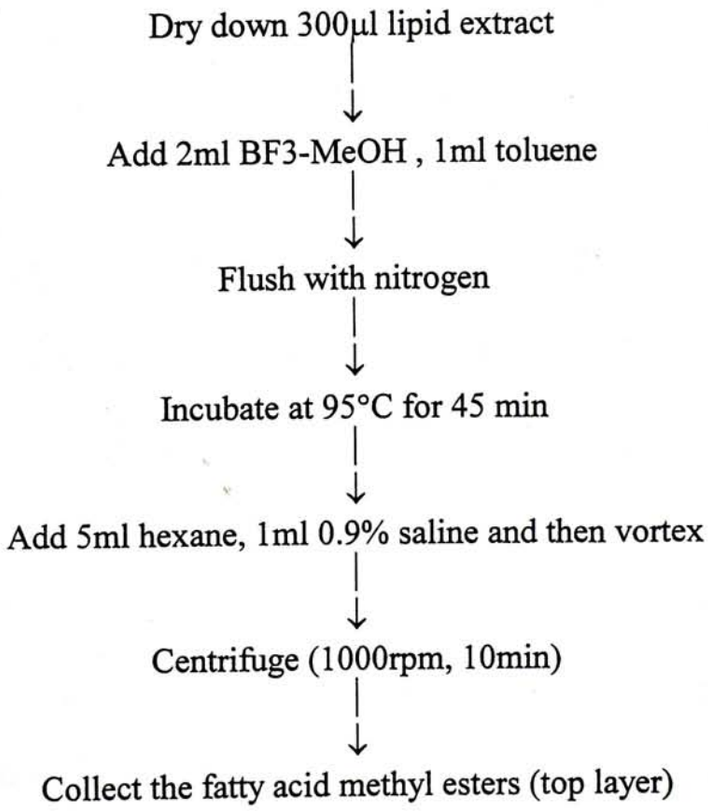


Figure 2.7 A flow chart of the methylation procedure.

The fatty acid methyl esters were analyzed by gas liquid chomatography (GLC) using a SP-2560 flexible fused silica capillary column (100m x 0.25mm, i.d., 20 μm film thickness; Supelco, Inc. Bellefonte, PA, USA) in a Hewlett-Packard 5980 Series II gas chomatograph, equipped with a flame ionization detector (Palo Alto, CA,USA). The column temperature was programmed from 180 to 220°C at a rate of 1°C/min and then held for 20 min. Injector and detector temperatures were set at 250°C and the column head pressure was set at 15 p.s.i. For the carcass samples, the fatty acid values were averaged from two aliquots and quantitated based on the amount of the IS added.

2.3.3 Triacylglycerol Species Analysis

The adipose tissue TG species were separated using an Alltech Model 525 HPLC (Deerfield, IL, USA) equipped with a ternary pump solvent delivery system and two reverse phase C-18 columns in series (Hypersil ODS, 250 x 4.6 mm, 5μm, Alltech, Deerfield, IL, USA; Microsorb MV, 250 x 4.6mm, 5μm, Rainin, Woburn, MA, USA) (Chen and Cunnane, 1993a). The adipose tissue TG samples (5-6μg) were injected onto the lead column via a rheodyne valve (20μL capacity, Alltech, Deerfield, IL, USA). A gradient of acetonitrile/chloroform was used at a flow rate of 0.8 ml/min (70:30 changing to 60:40 over 25 min held for 10 min and then back to 70:30 over 10 min). The separated TG species were monitored using an evaporative light scattering detector (Model MK III, Burtonsville, MD, USA) and a Hewlett Packard 3396 Series II integrator (Chen and Cunnane, 1993a). The detector temperature was set at 100°C. The pressure of the carrier gas was 1.8 p.s.i. TG species and their concentration were identified by comparing the retention time of adipose

tissue TG species with those of the known standards (Sigma Chemical, St. Louis, MO, USA) or the published TG species profiles of soybean, olive oil and corn oil. The individual TG species were designated by the combination of the first letter of the three fatty acids involved, for example, POO = OPO = OOP, which represents a triacylglycerol containing two oleic and one palmitic moieties regardless of the location of the three fatty acids esterified to glycerol.

2.3.4 Other Assays

Total serum triglycerides and serum cholesterol were determined by using enzymatic kits (Sigma Chemical, St. Louis, MO, USA).

2.3.5 Statistics

Data were expressed as means ± SD. Analysis of variance (ANOVA) followed by a least significance difference test was used for statistical evaluation of the significant difference between the CTL and WC rats and only $p<0.05$ was considered statistically significant. This was done by running the data on a PC ANOVA software (PC ANVOVA for the IBM Personal Computer, Version 1.1, IBM, Armonk, NY, USA).

2.4 Results

2.4.1 Food Intake

The time-course of food intake pattern is graphically illustrated in Figure 2.8. The food intake of the CTL groups throughout the entire experimental period was maintained at 29.5 ± 3.0g/rat/day. The weight reduction was achieved by giving no food for two days, 12.5g diet/rat/day for four days, and 18.5g/rat/day for eight days in experiment 1, experiment 2, experiment 3, respectively (Figure 2.8). When the WC rats were allowed free access to diet following the food restriction period, their food intake was slightly higher than that of the CTL group. This was clearly illustrated in experiment 3 (Figure 2.8) where each rat consumed 36.5g and 38.0g in the first refeeding day of cycle 1 and 2, respectively. Thereafter, the food intake gradually decreased and reached that of the CTL rats.

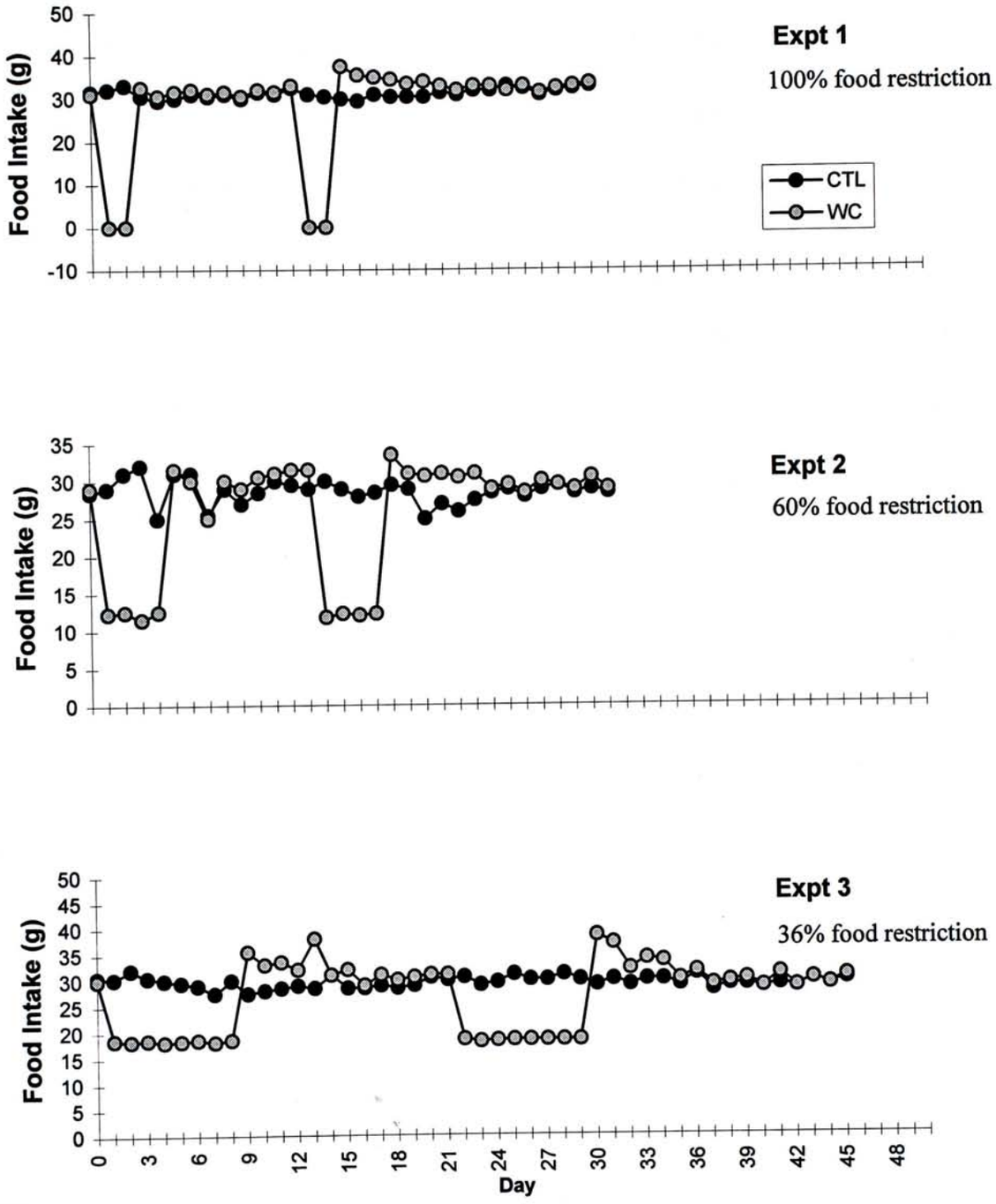


Figure 2.8 Food intake of rats in the three experiments.

2.4.2 Change of Body weight

The body weight of all WC rats dropped dramatically in the starting days of the restriction of the two WCs. After the first refeeding day in the two cycles, their body weight increased significantly and then gradually until they reached the same level as the CTL rats. After two consecutive WCs, the final body weight of the WC rats was not significantly different from that of the CTL.

In experiment 1, WC rats had a 48.5g and 39.0g weight reduction during the food restriction periods of cycle 1 and cycle 2, respectively. In cycle 1, the WC rats took a period of 10 days of *ad libitum* refeeding to reach the average weight of the CTL and they took 16 days in cycle2 (Figure 2.9).

In experiment 2, WC rats had a 42.5g weight reduction in cycle 1 and 35.5g weight reduction in cycle 2 when they were partially food restricted (60%). They took 9 days and 14 days of *ad libitum* refeeding to regain the weight lost during the food restriction period in WC 1 and WC 2, respectively (Figure 2.9).

WC rats in experiment 3 had a 27.0g and a 25.5g body weight reduction during 36% food restriction periods in WC 1 and WC 2, respectively. They took 13 days in WC 1 and 16 days in WC 2 to reach the average weight of the CTL (Figure 2.9).

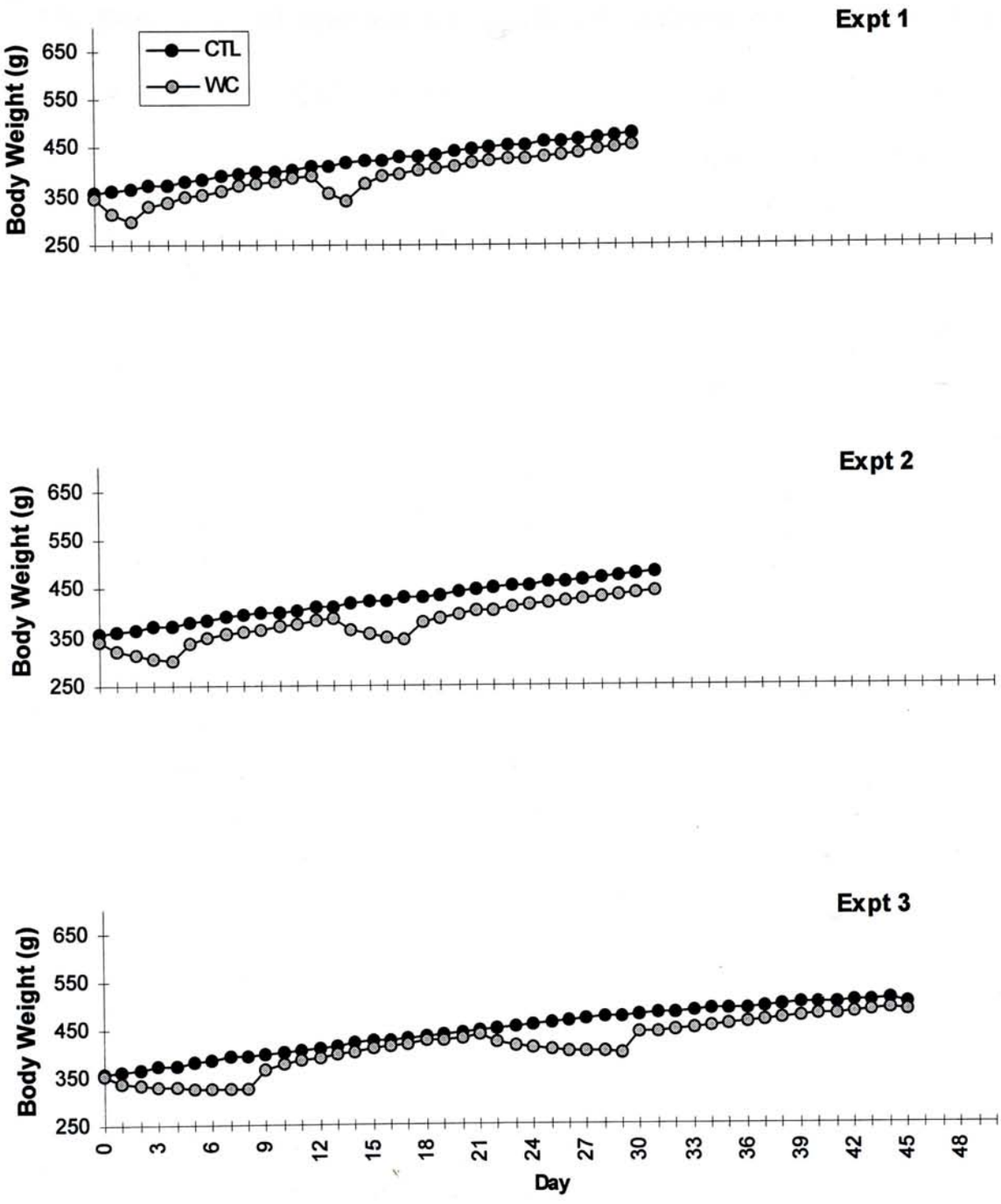


Figure 2.9 Change of the body weight during the 2 weight cycles in the experiments.

2.4.3 Weight of Liver and Adipose Tissues

The final weight of liver was not significantly different between the CTL and WC rats although it was slightly lower in the WC rats (Figure 2.10). There was no significant difference in the weight of epididymal and perirenal adipose tissue pads between the CTL and WC rats in all the three experiments (Figure 2.10)

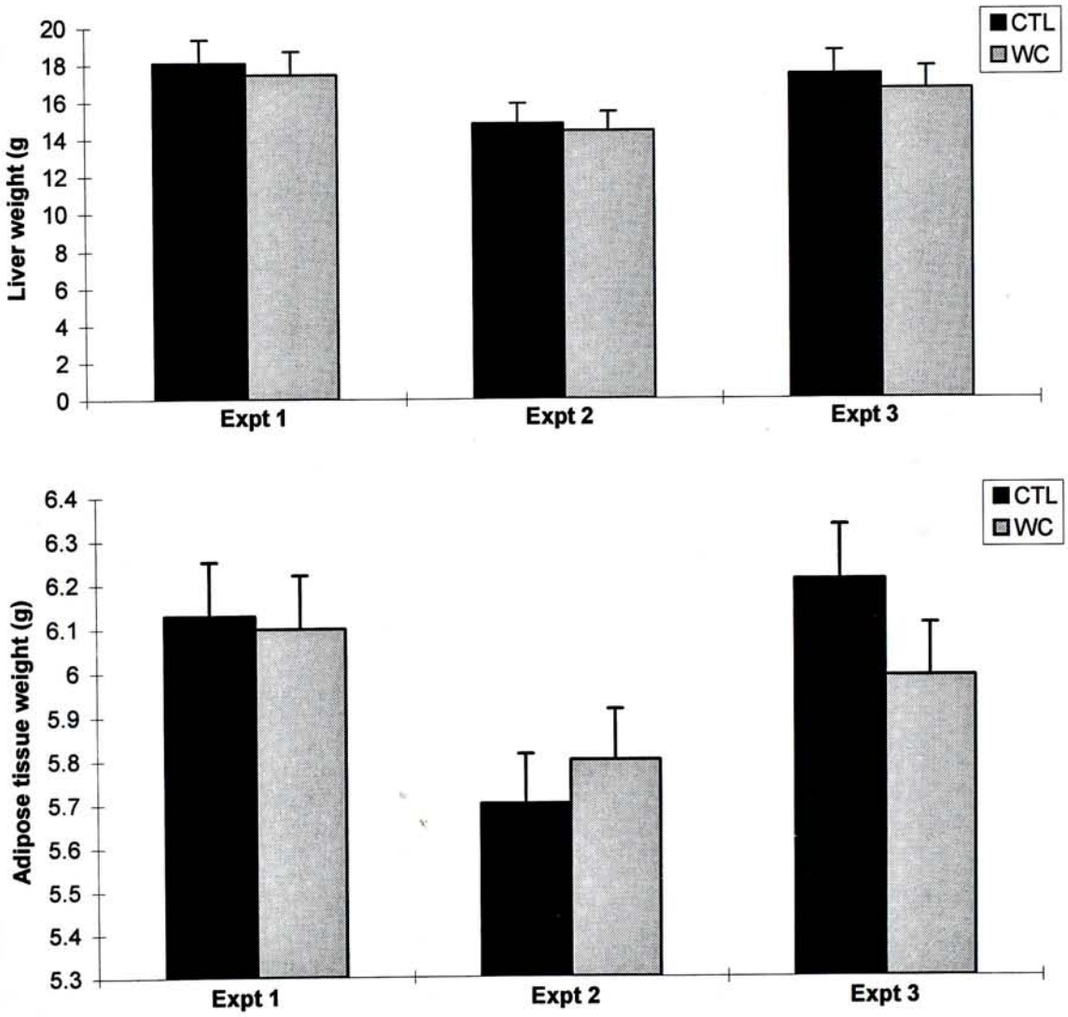


Figure 2.10 Weight of liver and total epididymal and perirenal adipose tissue of rats in the three experiments.

2.4.4 Serum Cholesterol and Triglycerides

Total serum cholesterol was not significantly different between the CTL and WC groups in all the three experiments (Figure 2.11). However, the total serum triglycerides were slighty reduced (20%-30%) in the WC rats compared with the corresponding CTL group in all the three experiments (Figure 2.12).

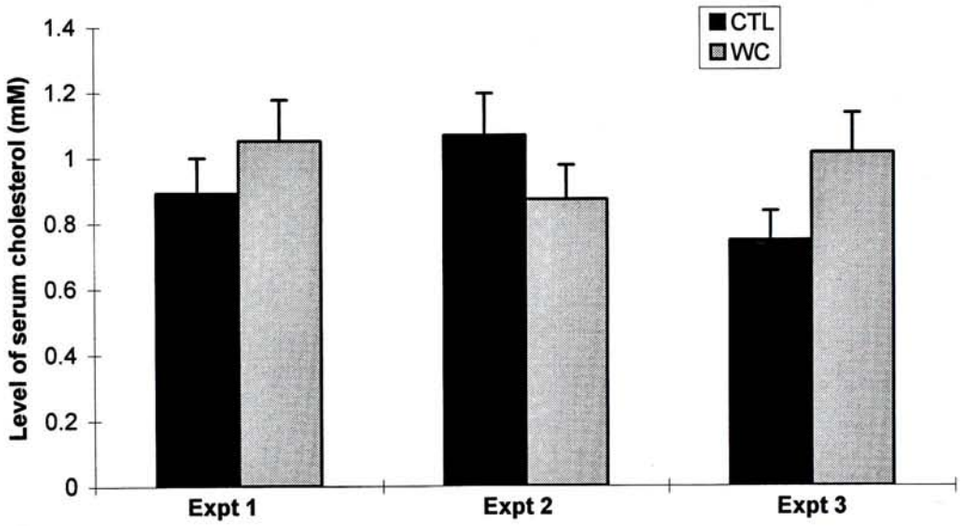


Fig. 2.11 Total serum cholesterol of rats in the three experiments.

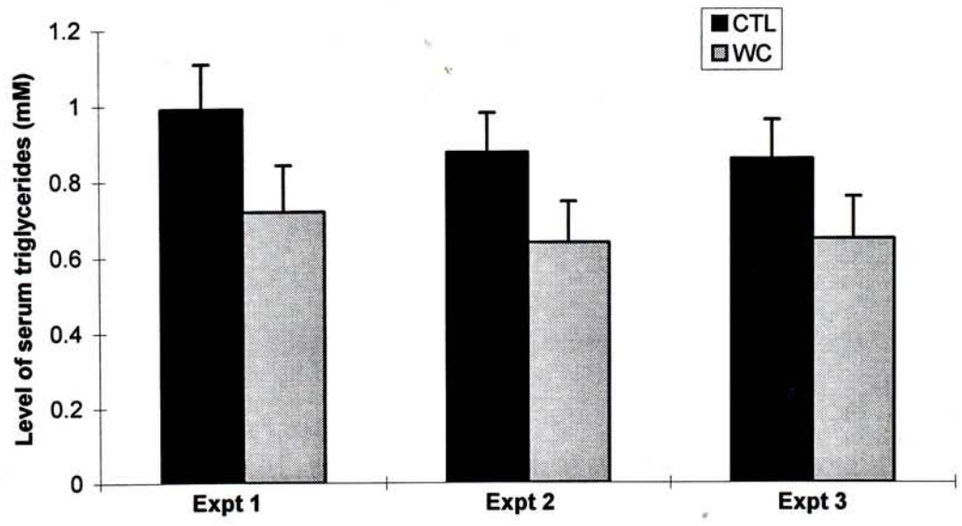


Figure 2.12 Total serum triglycerides of rats in the three experiments

2.4.5 Carcass Total Fatty Acids

The effect of weight cycling on the fatty acid composition of carcass is shown in Table.2.4. As a result of two WCs, the percentages of carcass myristic acid (14:0), 16:0, and16:1n-7 were significantly increased whereas the proportion of LA and α-LnA were significantly decreased in the WC rats when compared with the CTL (Figure 2.13). These observations were consistent in all the experiments ($p<0.01$). The two WCs did not change the proportion of 22:6n-3 except in experiment 1 where its proportion was lower in the WC rats than in the CTL group. Moreover, the ratio of SFAs to PUFAs increased in all the weight cycled rats (Figure 2.14).

Table 2.4 Effect of WC on fatty acid composition of carcass total lipids of rats in the three experiments

Fatty Acids	Experiment 1		Experiment 2		Experiment 3	
	CTL	**WC**	**CTL**	**WC**	**CTL**	**WC**
14:0	1.81 ±0.13	2.08 ±0.13**	1.71 ±0.10	2.31 ±0.52**	1.85 ±0.06	2.06 ±0.11**
16:0	22.36 ±0.76	25.16 ±0.45**	23.15 ±0.34	25.13 ±0.61**	22.70 ±0.29	24.77 ±0.73**
16:1n-7	3.66 ±0.57	4.38 ±0.37**	3.39 ±0.48	4.78 ±0.90**	3.60 ±0.12	4.74 ±0.75**
18:0	6.80 ±0.56	7.01 ±0.44	7.35 ±0.74	6.67 ±0.45	7.01 ±0.26	6.83 ±0.34
18:1n-9	31.69 ±0.54	32.51 ±0.18	31.5 ±0.73	32.23 ±0.39	32.15 ±0.49	32.36 ±0.41
18:2n-6	23.66 ±0.59	21.46 ±0.34**	23.86 ±0.80	20.59 ±0.52**	24.12 ±0.41	21.32 ±0.64**
20:4n-6	1.73 ±0.37	1.41 ±0.19	1.77 ±0.27	1.60 ±0.26	1.48 ±0.19	1.51 ±0.12
18:3n-3	1.60 ±0.10	1.29 ±0.06**	1.49 ±0.11	1.30 ±0.04**	1.50 ±0.08	1.36 ±0.03**
22:6n-3	1.23 ±0.18	0.97 ±0.12**	1.16 ±0.13	1.09 ±0.14	1.04 ±0.08	1.04 ±0.08
Total SFAs	31.66 ±0.51	34.94 ±0.36**	32.98 ±0.50	34.97 ±0.81**	32.35 ±0.42	34.31 ±0.83**
TotalMUFAs	36.40 ±0.86	37.61 ±0.47*	35.67 ±1.13	37.78 ±1.06**	36.67 ±0.61	37.91 ±1.10*
Total n-6	26.02 ±0.81	23.26 ±0.44**	26.21 ±1.03	22.64 ±0.70**	26.17 ±0.44	23.34 ±0.78**
Total n-3	3.32 ±0.17	2.57 ±0.21**	3.02 ±0.12	2.76 ±0.16*	2.88 ±0.10	2.73 ±0.17
Total FAs (mg/g)	6.99 ±1.24	6.82 ±0.58	6.94 ±0.63	7.48 ±1.07	7.55 ±0.49	7.24 ±0.74

Data are expressed as mean ± SD (n=6).
*$p<0.05$ and **$p<0.01$, between the control (CTL) and the weight cycled (WC) rats.

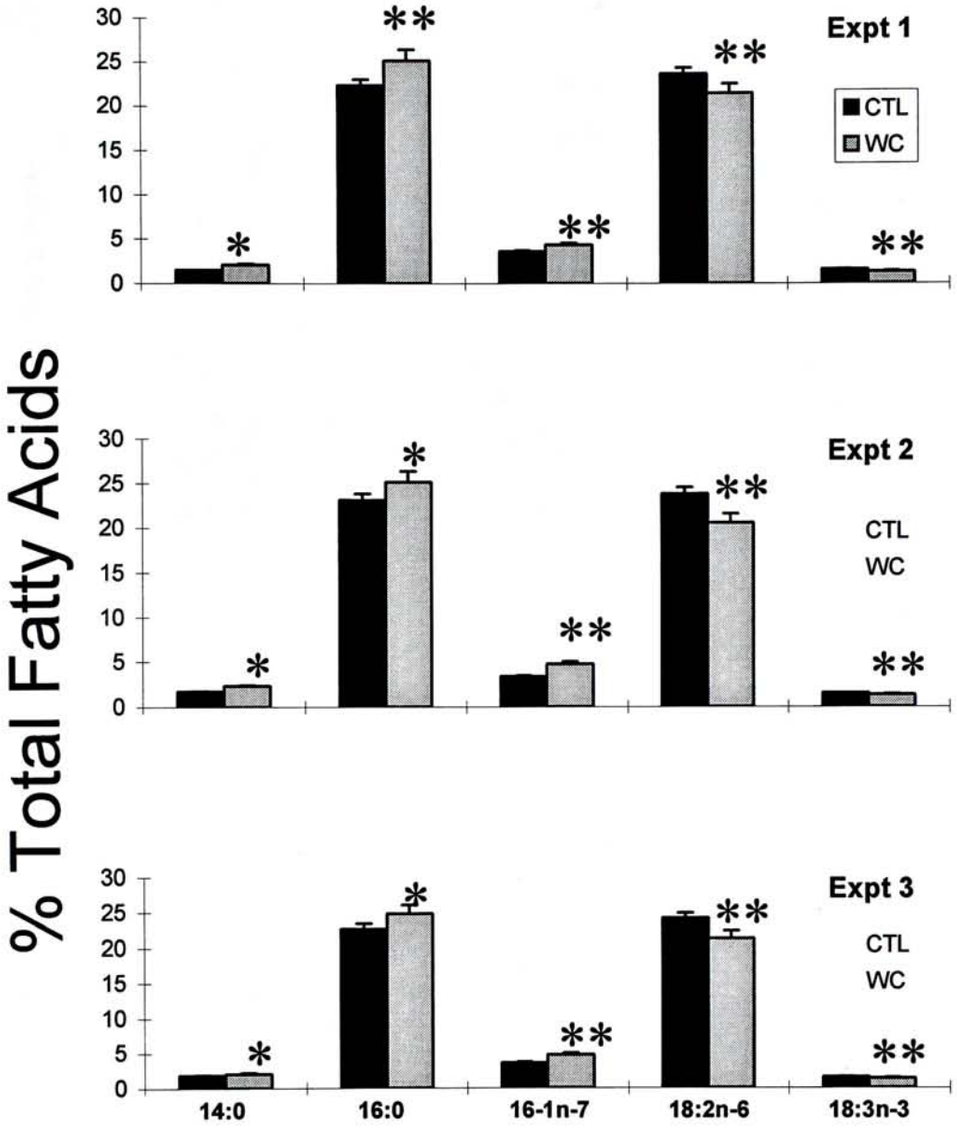


Figure 2.13 Fatty acid composition in carcass total lipids after 2 WCs in the three experiments. (*$p<0.05$ and **$p<0.01$, between the control (CTL) and the weight cycled (WC) rats.)

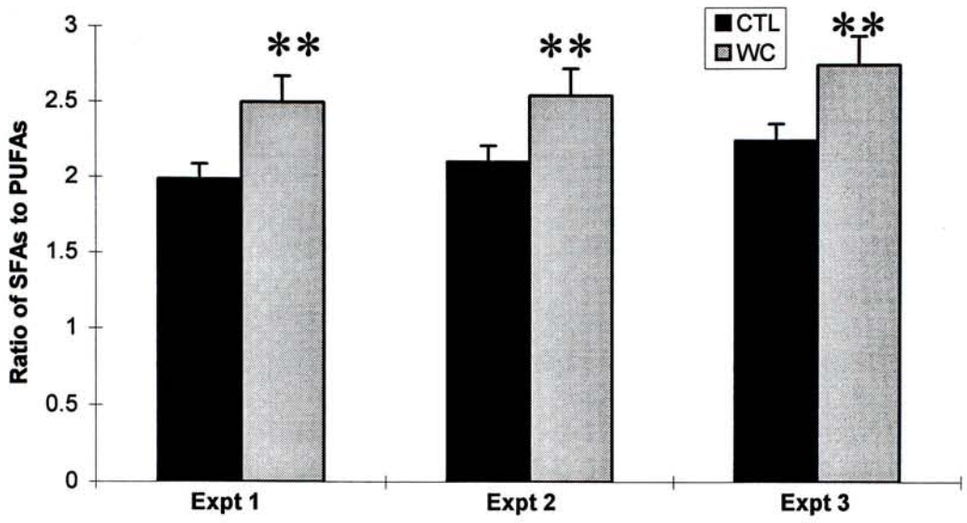


Figure 2.14 The ratio of SFAs to PUFAs in carcass total lipids after 2 WCs in the three experiments. (**p<0.01 between the control (CTL) and the weight cycled (WC) rats.)

2.4.6 Adipose Tissue Fatty Acids

Fatty acids in perirenal and epididymal adipose tissues responded similarly to weight cycling (Table 2.5). Similar to the changes of total carcasss lipids, 14:0, 16:0, and 16:1n-7 were proportionally increased whereas 18:2n-6 and 18:3n-3 were proportionally decreased in the adipose tissue of the WC rats compared with those of the CTL groups (Figure 2.15). There was no significant difference in the composition of other fatty acids between the CTL and WC rats in the three experiments.

Table 2.5 Effect of WC on fatty acid composition of adipose tissue of rats in the three experiments

Fatty Acids	Experiment 1		Experiment 2		Experiment 3	
	CTL	**WC**	**CTL**	**WC**	**CTL**	**WC**
14:0	1.92 ±0.15	1.78 ±0.06**	1.78 ±0.18	2.06 ±0.03**	1.67 ±0.09	1.89 ±0.05**
16:0	24.27 ±1.61	26.84 ±0.79**	24.49 ±1.14	27.40 ±1.20**	23.54 ±0.78	26.61 ±0.61**
16:1n-7	3.74 ±0.75	4.67 ±0.49**	3.56 ±0.84	5.63 ±0.58**	3.15 ±0.34	4.89 ±0.48**
18:0	5.72 ±0.45	5.71 ±1.04	5.99 ±0.64	5.99 ±0.26	6.26 ±0.28	5.75 ±0.37
18:1n-9	31.85 ±1.13	32.79 ±1.04	32.36 ±0.72	32.36 ±1.03	32.90 ±1.24	33.24 ±0.48
18:2n-6	24.88 ±1.52	21.36 ±1.52**	24.94 ±1.68	20.28 ±1.20**	25.67 ±0.85	20.90 ±0.99**
20:4n-6	0.42 ±0.07	1.39 ±0.05	0.42 ±0.05	0.38 ±0.05	0.38 ±0.02	0.43 ±0.03
18:3n-3	2.33 ±0.30	1.84 ±0.07**	2.12 ±0.11	1.81 ±0.07**	2.11 ±0.08	1.81 ±0.07**
22:6n-3	0.38 ±0.16	0.48 ±0.11	0.36 ±0.06	0.37 ±0.06	0.36 ±0.04	0.56 ±0.05**
Total SFAs	32.71 ±1.44	35.43 ±1.28**	33.08 ±0.64	35.45 ±0.99**	32.23 ±0.65	35.00 ±0.52**
Total MUFAs	36.29 ±1.06	38.17 ±0.80**	36.66 ±1.18	39.32 ±0.72**	36.81 ±0.48	38.87 ±0.89*
Total n-6	25.67 ±1.65	22.07 ±0.95**	25.85 ±1.68	21.00 ±1.20**	26.40 ±0.86	21.65 ±1.01**
Total n-3	2.94 ±0.28	2.53 ±0.24*	2.70 ±0.20	2.58 ±0.16	2.54 ±0.15	2.68 ±0.09

Data are expressed as mean ± SD (n=6).

*$p<0.05$ and **$p<0.01$, between the control (CTL) and the weight cycled (WC) rats.

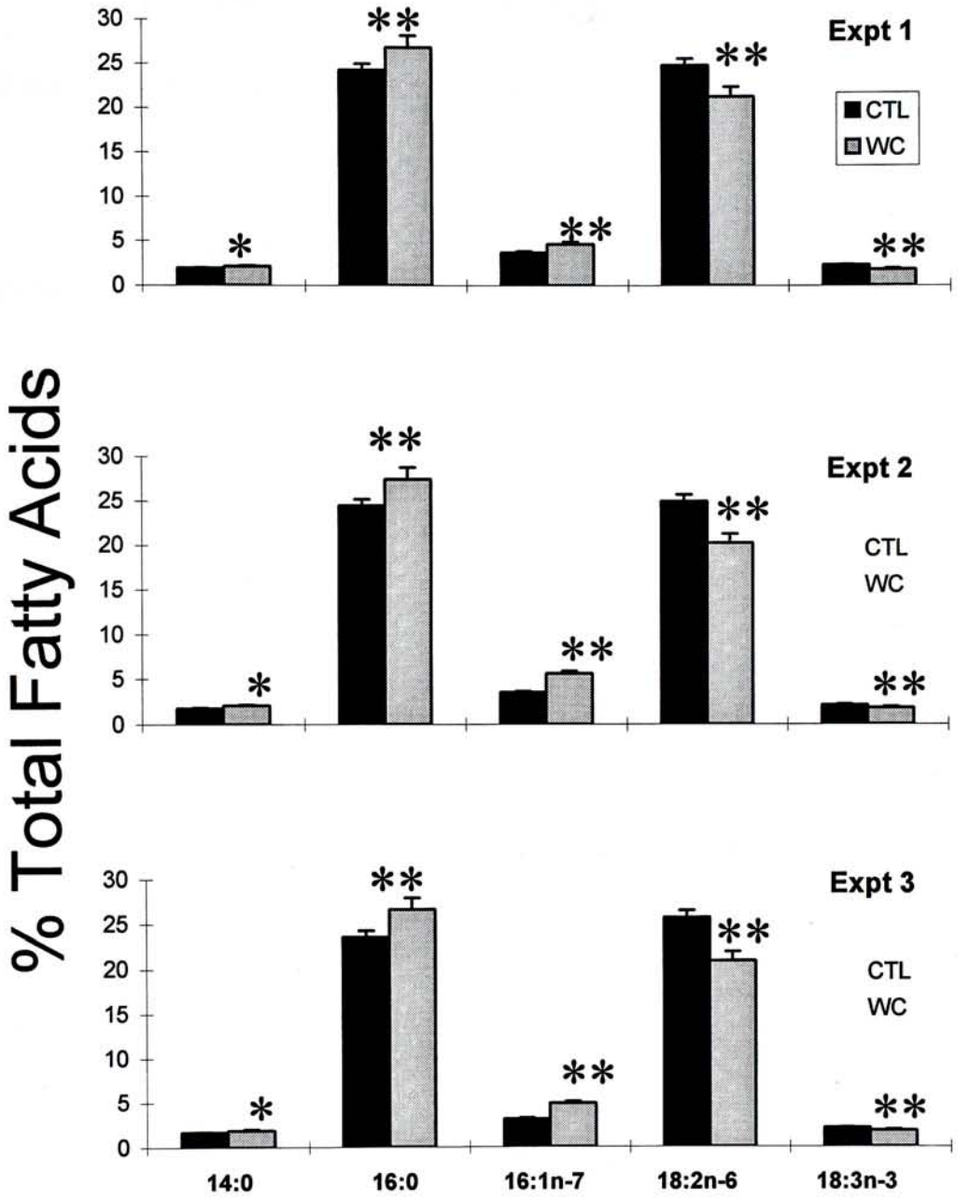


Figure 2.15 Fatty acid composition in adipose tissue after 2 WCs in the three experiments. (*$p<0.05$ and **$p<0.01$, between the control (CTL) and the weight cycled (WC) rats.)

2.4.7 Liver Fatty Acids

At the end of WC 2, all the individual liver fatty acids in the WC rats did not show any significant difference from those in the CTL rats (Table.2.6)

Table 2.6 Effect of WC on liver fatty acid composition of rats in the three experiments

Fatty Acids	Experiment 1		Experiment 2		Experiment 3	
	CTL	**WC**	**CTL**	**WC**	**CTL**	**WC**
14:0	0.32 ±0.06	0.30 ±0.06	0.26 ±0.07	0.28 ±0.04	0.18 ±0.05	0.23 ±0.04
16:0	19.75 ±0.89	19.53 ±0.06	20.01 ±1.75	20.55 ±1.54	19.25 ±1.95	19.92 ±0.98
16:1n-7	0.97 ±0.20	0.93 ±0.31	0.82 ±0.27	1.12 ±0.33	0.92 ±0.30	1.08 ±0.37
18:0	17.69 ±1.54	18.85 ±1.65	18.87 ±1.64	18.21 ±1.88	16.72 ±2.70	16.73 ±2.05
18:1n-9	12.26 ±1.10	11.33 ±1.13	11.71 ±1.62	12.53 ±1.21	12.29 ±2.14	14.73 ±2.07
18:2n-6	17.64 ±0.94	17.67 ±1.58	17.16 ±0.81	17.43 ±0.76	18.57 ±1.32	18.60 ±0.97
20:4n-6	17.60 ±1.77	17.88 ±0.98	18.47 ±2.67	16.89 ±1.72	17.65 ±2.87	16.10 ±2.00
18:3n-3	0.42 ±0.19	0.28 ±0.09	0.35 ±0.16	0.35 ±0.16	0.35 ±0.06	0.31 ±0.12
22:6n-3	5.85 ±0.25	5.71 ±0.61	5.78 ±0.63	5.31 ±0.51	5.84 ±0.55	5.81 ±0.05
Total SFAs	39.65 ±1.61	41.26 ±2.08	40.52 ±1.20	40.79 ±2.00	37.86 ±0.55	38.47 ±1.23
Total MUFAs	13.54 ±1.55	12.41 ±1.18	12.73 ±2.19	13.83 ±1.93	14.45 ±2.62	16.05 ±2.40
Total n-6	37.05 ±1.09	37.30 ±2.13	37.49 ±2.34	36.10 ±1.59	37.96 ±2.68	36.05 ±1.47
Total n-3	8.28 ±0.62	7.46 ±0.63	7.71 ±0.74	7.83 ±0.80	8.23 ±0.50	7.95 ±0.86

Data are expressed as mean ± SD (n=6).

2.4.8 Adipose Tissue TG Species

The adipose tissue of WC rats had a TG species profile distinctly different from that of the CTL (Figure 2.16). As shown in Table 2.7, 16:0 - or 16:1n-7 - enriched TG species including OPPo, PPPo and PPP were significantly increased in the WC rats compared with those in the CTL ($p<0.01$). In contrast, 18:2n-6 - or 18:3n-3 - enriched TG species including LLL, LnLnS, LLO and LLP were proportionally depleted in the WC rats compared with those in the CTL ($p<0.01$). The

other two TG species, LOP and OOP, were also decreased in the WC rats compared with these in the CTL group (Figure 2.17).

1. LLL
2. LLnO
3. LnLnS
4. LLnP
5. LLO
6. LnOO
7. LLP
8. LnOP
9. LnPP
10. LOO
11. LLS
12. LOP
13. OPPo
14. LPP
15. PPPo
16. OOO
17. LOS
18. OOP
19. OPP
20. PPP
21. OPS
22. LSS
23. PPS
24. SSS

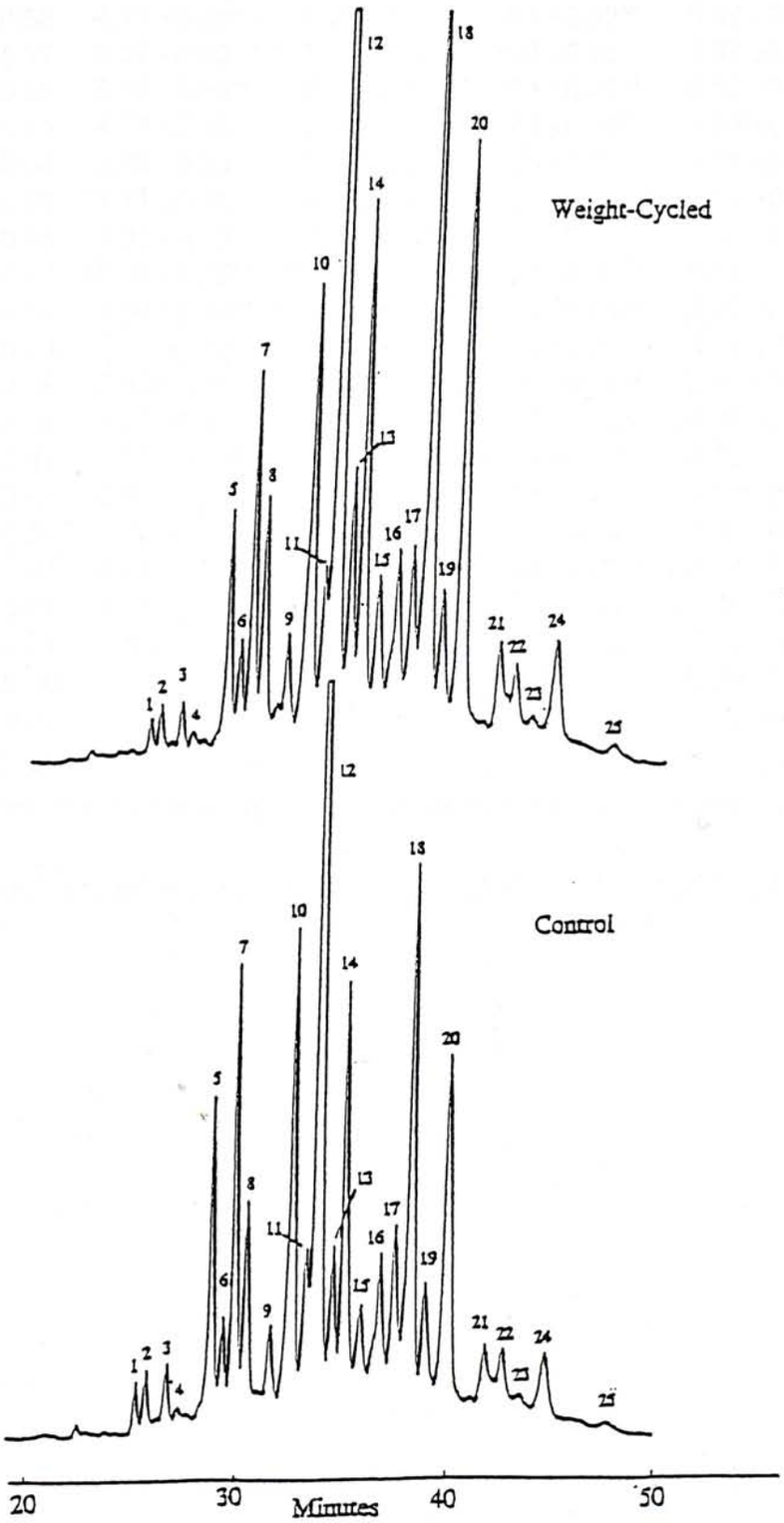


Figure 2.16 TG profiles of the weight-cycled rat and the control rat.

Table 2.7 Effect of WC on adipose TG species of rats in the three experiments.

TG Species@		Experiment 1		Experiment 2		Experiment 3	
		CTL	**WC**	**CTL**	**WC**	**CTL**	**WC**
1.	LLL	1.02 ±0.18	0.63 0.07**	0.97 ±0.23	0.57 ±0.10**	0.96 ±0.10	0.56 ±0.11**
2.	LLnO	1.15 ±0.09	0.86 0.07**	1.12 ±0.07	0.83 ±0.06**	1.04 ±0.05	0.81 ±0.13*
3.	LnLnS	1.09 ±0.09	0.79 ±0.22*	1.15 ±0.13	0.81 ±0.10**	1.05 ±0.08	0.76 ±0.28*
4.	LLnO	0.35 ±0.04	0.42 ±0.10	0.36 ±0.05	0.43 ±0.06	0.36 ±0.02	0.38 ±0.17
5.	LLO	5.45 ±0.58	4.15 ±0.28**	5.62 ±0.75	3.61 ±0.52**	5.68 ±0.37	4.01 ±0.39**
6.	LnOO	1.98 ±0.17	2.02 ±0.09	1.97 ±0.20	2.09 ±0.12	1.82 ±0.13	2.00 ±0.15
7.	LLP	6.41 ±0.36	5.16 ±0.28**	6.55 ±0.57	4.83 ±0.40**	6.50 ±0.49	5.13 ±0.33**
8.	LnOP	3.74 ±0.35	4.07 ±0.15	3.74 ±0.14	4.28 ±0.26?	3.50 ±0.38	4.08 ±0.31
9.	LnPP	1.95 ±0.60	2.70 ±0.29	1.78 ±0.39	2.38 ±0.74	1.74 ±0.38	2.45 ±0.60
10.	LOO	8.71 ±0.80	7.71 ±0.32	8.90 ±0.82	7.38 ±0.60**	8.87 ±0.23	7.28 ±0.28**
11.	LLS	3.50 ±0.46	3.02 ±0.13	3.19 ±0.13	3.35 ±0.25	3.26 ±0.11	3.10 ±0.08
12.	LOP	18.19 ±0.47	17.00 ±0.75**	18.44 ±0.32	16.53 ±0.57**	18.55 ±0.46	17.65 ±0.27**
13.	OPPo	3.34 ±0.51	4.34 ±0.44**	3.22 ±0.74	5.18 ±0.54**	2.88 ±0.30	4.60 ±0.42**
14.	LPP	7.11 ±0.23	7.11 ±0.27	7.31 ±0.10	6.96 ±0.31	7.19 ±0.13	6.98 ±0.30
15.	PPPo	2.64 ±0.38	3.40 ±0.16**	2.52 ±0.34	3.76 ±0.31**	2.46 ±0.17	3.44 ±0.19**
16.	OOO	4.22 ±0.35	4.27 ±0.23	4.24 ±0.31	4.14 ±0.40	4.38 ±0.13	4.02 ±0.16
17.	LOS	4.09 ±0.44	3.67 ±0.32	4.14 ±0.58	3.36 ±0.27*	4.42 ±0.27	3.42 ±0.33**
18.	OOP	9.58 ±0.81	10.98 ±0.63*	9.17 ±1.02	11.80 ±0.77**	9.54 ±0.63	11.53 ±0.46**
19.	OPP	2.83 ±0.24	2.72 ±0.21	2.87 ±0.31	2.55 ±0.19	2.99 ±0.14	2.59 ±0.22
20.	PPP	6.73 ±0.81	8.42 ±0.51**	6.91 ±0.98	8.93 ±0.77**	6.57 ±0.54	8.75 ±0.33**
21.	OPS	1.39 ±0.27	1.77 ±0.40	1.35 ±0.64	2.00 ±0.44	1.46 ±0.24	1.82 ±0.31
22.	LSS	1.49 ±0.71	1.43 ±0.46	1.17 ±0.25	1.23 ±0.32	1.58 ±0.54	1.23 ±0.17
23.	PPS	0.22 ±0.10	0.25 ±0.12	0.18 ±0.07	0.23 ±0.09	0.24 ±0.05	0.18 ±0.08
24.	SSS	2.13 ±0.24	2.35 ±0.24	1.81 ±0.14	2.00 ±0.19	2.21 ±0.22	2.40 ±0.32

Data are expressed as mean ± SD (n=6).

@Some of them may overlap with the TG species containing 20:4n-6, 22:6n-3 and 14:0.

*$p<0.05$ and **$p<0.01$, between the control (CTL) and the weight cycled (WC) rats.

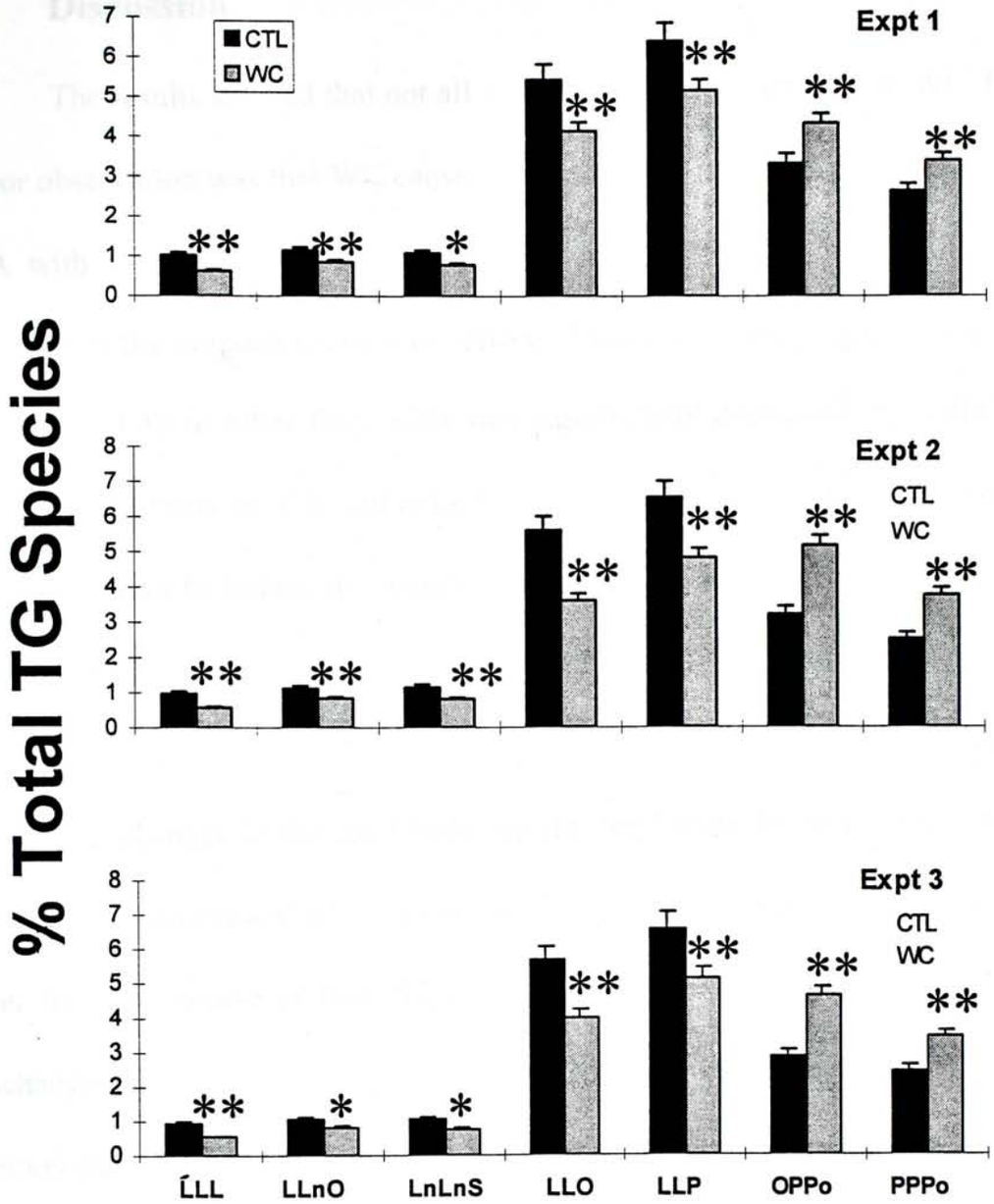


Figure 2.17 Effect of WC on changes in adipose TG species of rats in the three experiments. (*$p<0.05$ and **$p<0.01$, between the control (CTL) and the weight cycled (WC) rats.)

2.5 Discussion

The results showed that not all fatty acids respond similarly to WC. Firstly, the major observation was that WC caused a proportional depletion of PUFAs, LA and α-LnA with a concomitant increase in SFAs and MUFAs, including 14:0, 16:0 and 16:1n-7, in the adipose tissue and carcass (Figure 2.13 and Figure 2.15). Thus, the ratio of PUFAs to other fatty acids was significantly decreased. Secondly, the WC-induced reductions of LA and α-LnA were specific regardless of the extent of food restriction used to induce the weight reduction. The adipose tissue and carcass fatty acid composition was remodelled significantly by 36-100% food restriction followed by the *ad libitum* refeeding. However, this fatty acid remodelling occurred without significant changes in the total body weight, total body fat and adipose tissue pads. Thirdly, the carcass and adipose fatty acid composition were significantly remodelled over the time course of two WCs although the dietary fatty acid profile remained unchanged throughout the study and the food was available to the WC rats on *ad libitum* basis during the refeeding. Chen *et al.* (1995) showed that LA and α-LnA were still depleted in young growing weight-cycled rats even there was an adequate supply of n-6 and n-3 PUFAs in the diet during the refeeding periods. Thus, together with the previous reports, the specific reduction in LA and α-LnA induced by weight cycling was independent of the extent of calorie restriction, the pattern of the weight cycling, the animal age, as well as the dietary levels of LA (Chen *et al.*, 1995; 1996).

The mechanism by which LA and α-LnA are selectively depleted while 14:0, 16:0 and 16:1n-7 are proportionally increased during weight cycling remains unknown. However, it is possible that LA and α-LnA are preferentially mobilized

from adipose tissue and then oxidized in mitochondria during food restriction. Raclot and Groscolas (1993) demonstrated that under stimulated lipolysis, the individual fatty acids were more readily mobilized from fat cells if they were relatively short and unsaturated. As shown in Figure 2.18, LA and α-LnA are preferentially mobilized compared with 14:0 and 16:0. Leyton *et al.* (1987) have also shown that LA and α-LnA are two of the most readily oxidized fatty acids *in vivo* (Figure 2.19).

Furthermore, by using the whole body fatty acid balance technique, Chen *et al.* (1996) showed that repeated fasting followed by refeeding for four cycles apparently caused higher oxidation of LA and α-LnA compared with the *ad libitum* feeding. To be specific, 66% dietary LA and 78% dietary α-LnA in the *ad libitum* fed control rats were oxidized whereas, in the rats with repeated fasting and refeeding, 82% dietary LA and 88% dietary α-LnA were apparently oxidized.

During subsequent refeeding, the lipogenesis of saturated and monounsaturated fatty acids was probably stimulated in the liver and adipose tissue and contributed to the higher concentration of 14:0, 16:0 and 16:1n-7 in serum. This was in agreement with the observation that refeeding with a fat-free diet for 23h after a 48 h period of starvation increased incorporation of [1-^{14}C] acetate into 16:0 by 2-fold, into 16:1n-7 by 8-fold, into 18:0 by 3-fold, and into 18:1n-9 by 24-fold (Allman *et al.,* 1965). In addition, LA and α-LnA have been suggested to inhibit lipogenesis (Reid and Husbands, 1985) and thus their decrease may indirectly lead to the synthesis of SFAs and MUFAs (Cunnane, 1996).

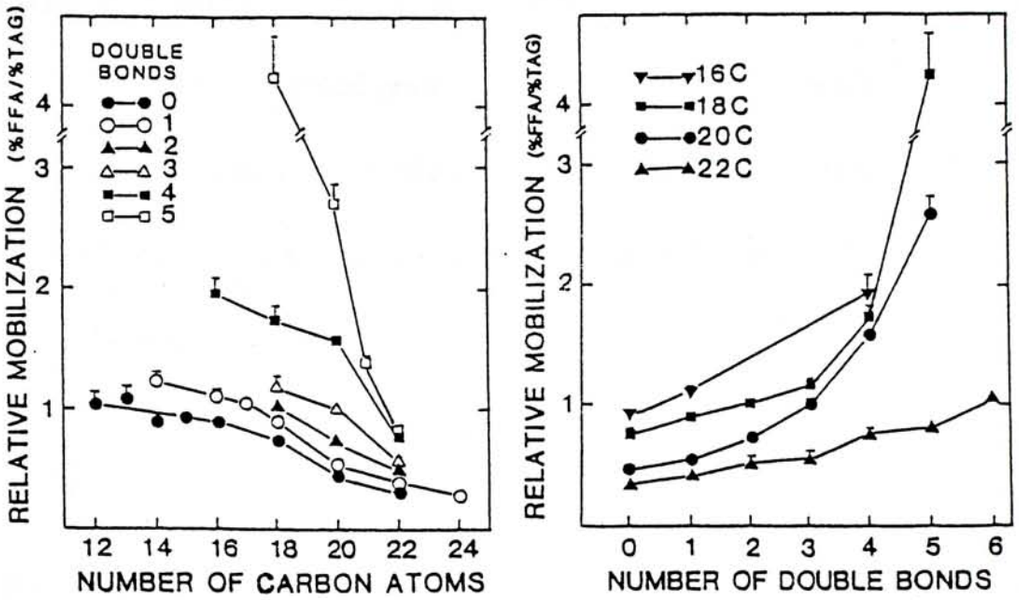


Figure 2.18 Differential mobilization of saturated and unsaturated fatty acids (Raclot and Groscolas, 1993).

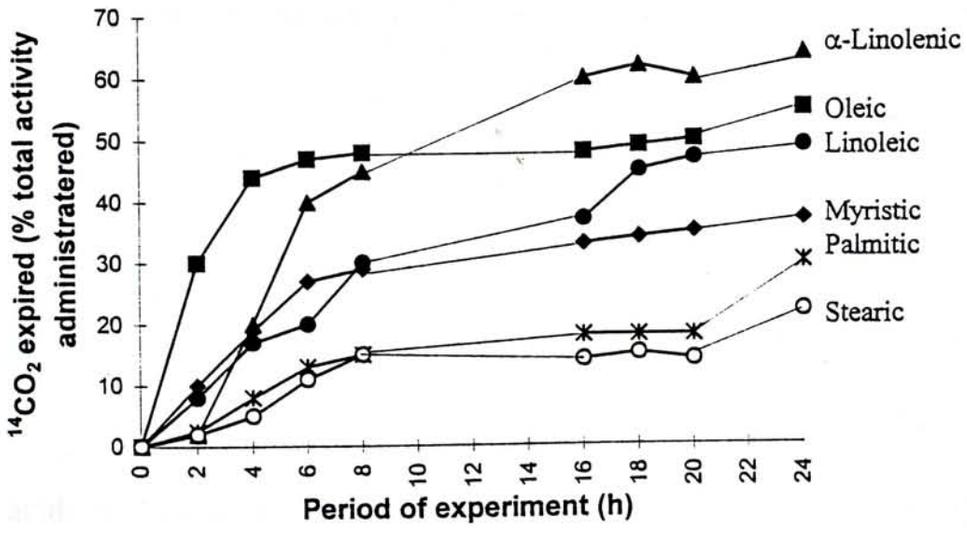


Figure 2.19 Differential oxidation of saturated and unsaturated fatty acids in mitochondria. The *in vivo* oxidation of 5-6 μCi ^{14}C-labelled fatty acid in 0.2 ml olive oil, was put into a metabolism chamber and the expired $^{14}CO_2$ were collected and measured.(Leyton *et al.*, 1987).

Thus, LA and α-LnA were preferentially mobilized from the adipose tissue and were then readily oxidized in mitochondria during the food restriction. On the other hand, more saturated and monounsaturated fatty acids were subsequently synthesized during *ad libitum* refeeding. As a result, lesser amount of LA and α-LnA but more saturated and monounsaturated fatty acids were diverted to triglyceride synthesis in adipose tissue.

To test this hypothesis, the present result showed that the ratio of SFAs to PUFAs was increased after the two WCs (Figure 2.14). Moreover, the individual adipose tissue TG species were separated and quantitated. The results demonstrated clearly that two WCs remodelled the composition of adipose tissue with a selective accumulation of OPPo, PPPo and PPP species but a preferential depletion of LLL, LnLnS, LLO and LLP species. This suggests that less saturated and monounsaturated enriched TG species but more polyunsaturated enriched TG species are cleaved, mobilized and oxidized during the food restriction, or more saturated and monounsaturated TG species but less polyunsaturated TG species were synthesized during refeeding after a period of food restriction.

As mentioned in Chapter 1, WC may increase the risk of CHD. If so, part of the mechanism may be due to the selective depletion of LA and α-LnA reserves and the subsequent changes in the balance between the saturated and polyunsaturated fatty acids in various tissues including muscle, adipose tissue and circulating lipid pools (Brownell *et al.,* 1986; Borkman *et al.,* 1993).

It remains controversial whether WC can increase the body fatness. Most studies of weight cycling were focused on the effects of refeeding after fasting on total body fat, food efficiency, and fat consumption. In obese humans, a single WC neither led to a greater body fatness nor to a larger amount of visceral fat compared with that before weight loss (Kooy *et al.,* 1993). Although two to three cycles of weight loss and weight regain resulted in the consumption of a higher percentage of dietary fat, increased food efficiency, increased adiposity and hyperinsulinemia in rats, this diet-induced adiposity occurred with a free selection of diets during the refeeding or refed with a high fat diet (Bjorntorp *et al.,* 1980; Brownell *et al.,* 1986). The present study demonstrated that feeding with rodent chow diet, two WCs changed the fatty acid composition. However, weight cycling did not produce an increase in body fatness in rats fed a chow diet although there was a slight overshoot of food intake on the first day of refeeding after a period of food restriction (Figure 2.8).

Chapter 3

Influence of Dietary Fat Level on Fatty Acid Composition and Adiposity in Weight-Cycled Rats

3.1 Introduction

3.1.1 Fat Preference and Intake in Humans

There is a general consensus that fat intake should be controlled to help maintaining an active and healthy lifestyle. Moreover, moderate fat intake has been a public health recommendation for more than 75 years (U.S. Department of Health and Human Services, 1988). Despite the repeated recommendations from nutritionists and the widespread concern by health professionals and the public about the potential adverse health effects of a high-fat diet, the consumption of dietary fat still remains high (Stephen and Wald, 1990). This may be due to the fact that fat makes an important contribution to the desirable texture and flavor of many food products, and these favorable properties cannot be replaced by their low-fat or very low-fat counterparts. Moreover, the low satiating power of fats (Blundell and Burley, 1990) and their high energy density (9 kcal/g, as opposed to 4 kcal/g for proteins and carbohydrates) can also lead to an excessive of total energy.

It is believed that high-fat diet promotes obesity. Some interesting findings were extensively reported by Drewnowski and his colleagues (1985). It was found that the fondness for fats among obese persons was markedly higher than that among persons of normal weight. Preferences for fat-rich foods among obese individuals are

confirmed by studies based on different methodologies. For instance, Drewnowski *et al.* (1992) demonstrated that obese persons preferred very fatty stimuli (at least 34% fat) compared with the control group (20%).

Recent studies also emphasize that the preferred food of bulimic individuals (Hadigan *et al.,* 1989) and patients with binge-eating disorder (Yanovski *et al.,* 1992) are still fatty foods. Furthermore, high-fat foods are also preferentially desired by individuals in negative energy balance (in starvation or in dieting). Findings from the Minnesota starvation experiment indicate that when released from a period of severe energy restriction, participant cravings were primarily directed toward high-fat items, such as ice-cream, pastries, cheese and nuts (Keys *et al.,* 1950). In a study performed by Drewnowski and Holden-Wiltse (1992), it was also demonstrated that after slimming, formerly obese individuals showed a strong partiality of fat. Therefore, weight cycling or "yo-yo" syndrome is one of the changes caused by repeated dieting and collapse or switching from a low-fat to a high-fat diet or vice versa in obese individuals. These changes and the fat preferences in humans are influenced by genetic predisposition (deCastro, 1993), neural mechanisms (Young *et al.,* 1988; Cooper, 1983), metabolic response (Sclafani and Ackeroff, 1993), as well as cognitive and sensory factors (Shepherd and Stockley, 1987; Lloyd *et al.,* 1995).

3.1.2 Alteration of Lipid Metabolism Induced by Dietary Fat

It is well established that the level of dietary fat and the fatty acid composition of diet influence lipid metabolism in adipose tissue (Lhuillery *et al.,* 1988; Hwang *et al.,* 1992). They are also important factors in determining body weight and energy stores (Hill *et al.,* 1992).

Due to the high energy density and weak satiety effect of fat, excessive consumption of fats does not lead to high oxidation of fats, and therefore the surplus fats are stored in the body's reserves (Verboeket-van deVenne *et al.,* 1994). Although high-fat diet suppresses hepatic lipogenesis (Herzberg and Rogerson, 1988), it does not affect lipogenesis in adipose tissue (Herzberg and Rogerson, 1988; Nelson, *et al.,* 1987). On the contrary, high-fat diet promotes weight gain, body fat accumulation (Wood and Reid, 1975; Donato and Hegsted, 1985; West *et al.,* 1992) and other chronic diseases (Vanderveen, 1995). Boozer *et al.* (1995) have demonstrated that total body fat and adipose depot weights increased in proportion to the level of dietary fat in rats fed isocalorically (Figure 3.1). Furthermore, a recent study has noted that a high-fat diet brought about changes in body fatty acid composition in rats (Llado *et al.,* 1996).

Not only the quantity, but also the quality of dietary fat also plays a key role in the regulation of lipid metabolism (Gaiva Gomes daSilva *et al.,* 1996). Diets rich in PUFAs are more effective than those rich in SFAs in suppressing the activities of lipogenic enzymes and their mRNA expression (Clarke *et al.,* 1990; Cheema and Clandinin, 1996) (Figure 3.2). In addition, according to Beynen and Katan (1985),

PUFAs promote oxidation of dietary fatty acids, whereas SFAs like 16:0 promotes lipoprotein synthesis and lipid storage.

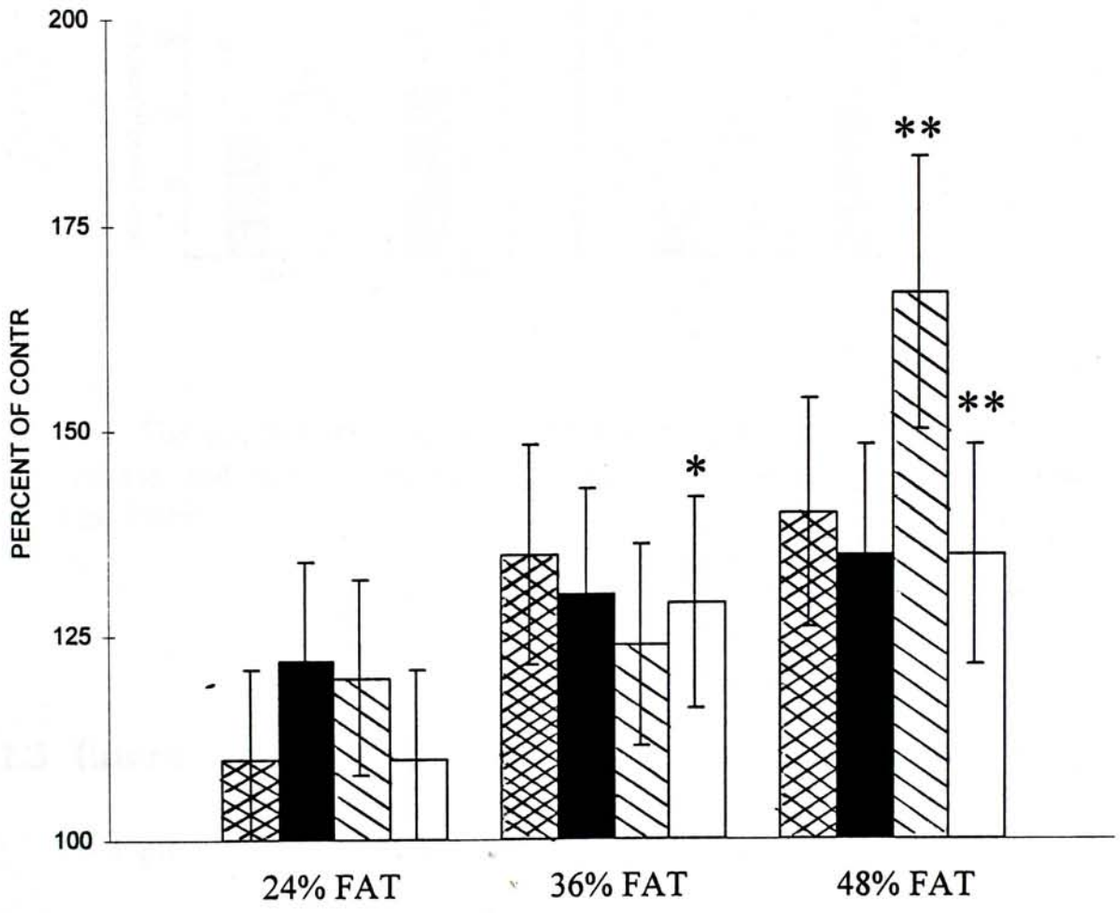


Figure 3.1 Adipose depots in comparison with controls increase in a dose response manner with increasing proportion of dietary energy from fat (*$p<0.05$ and **$p<0.01$). Cross-hatched bars, epididymal; filled bars, retroperitoneal; hatched bars, mesenteric; open bars, total adipose tissue (Boozer *et al.*, 1995).

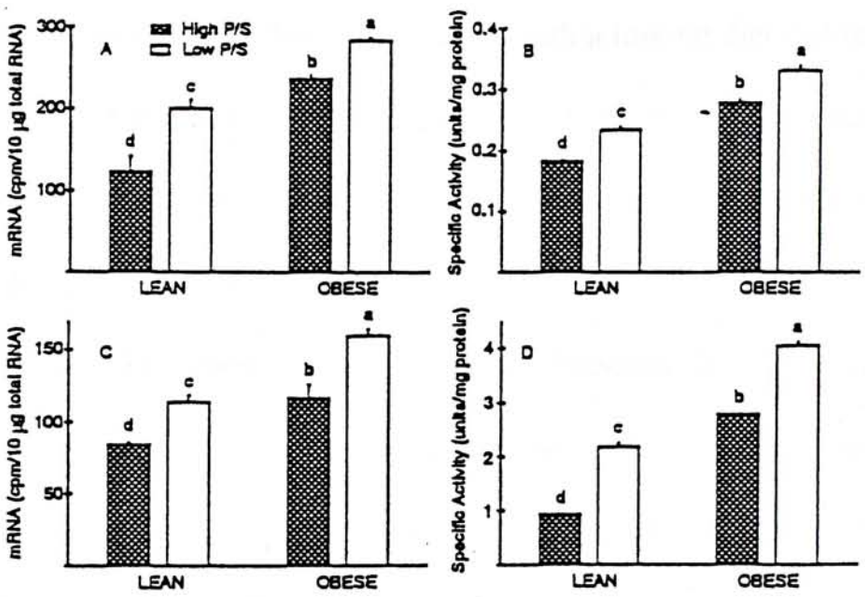


Figure 3.2 The relative abundance of mRNA and specific activity of hepatic fatty acid synthase and acetyl CoA carboxylase in lean and obese rats (Cheema and Clandinin, 1996).

3.1.3 Interaction Between Weight Cycling and Fat Intake

Weight cycling experiments often involve changes in the composition of food, as well as changes in the amount of food. Some effects on energy utilization attributed to weight cycling may be the effects of changes in diet composition, or of the interaction between weight cycling and a change in diet composition. A series of studies demonstrated that weight cycling with a constant low-fat diet did not affect energy efficiency, energy expenditure and body composition (Hill *et al.*, 1987; 1988; Wheeler *et al.*, 1990).

On the other hand, a study carried out by Uhley and Jen (1989) reported that weight cycling, produced by alternating *ad libitum* consumption of high-fat and low-fat diets, resulted in an increase of food efficiency. In contrast, no such increase was found when rats were first subjected to 3 WCs with a low-fat diet and then allowed *ad libitum* access to a high-fat diet (Graham, 1990). Furthermore, in a study by Reed *et al.* (1993), both *ad libitum* control and weight-cycled rats were allowed to have a choice of 3 mixed diets which differed in the proportion of calories derived from carbohydrate and fat. There was no difference between these two groups in body weight, body fatness, dietary fat intake, central adiposity and food efficiency. However, Reed *et al.* (1993) reported only the total food intakes in 2 and 4 weight cycles but not the food intake of weight cycled rats during the refeeding periods. Furthermore, the food efficiency that they defined (the difference between the initial and the final weight in the experiment in gram divided by total food intake in the experiment in gram) could not reflect reality as the change of body weight in weight-cycled rats gave negative value during fasting periods. So, a comparison of food efficiency at particular period of weight cycling would be more informative.

3.2 Objective of the Present Study

The level of dietary fat influences lipid metabolism in adipose tissue (Lhuillery *et al.,* 1988; Hwang *et al.,* 1992). Lipid metabolism is one of the important factors in determining body weight and energy stores (Hill et al, 1992). Most weight cycling experiments applied constant low fat diets and did not demonstrate any effect on energy utilization (Hill *et al.,* 1987; 1988; Wheeler *et al.,* 1990). High-fat diets were also applied in some weight cycling experiments (Uhley and Jen, 1989; Graham *et al.,* 1990; Reed *et al.,* 1993). However, the high-fat diet was used only in certain periods rather than throughout the entire experiment.

This study was conducted in order to investigate the differences between a constant high-fat diet and a medium-fat diet in body fatty acid composition of WC rats. In the previous study (Chapter 2), weight cycling induced by different extent of calorie restriction altered the fatty acid balance with a preferential depletion of LA and α-LnA. However, the diet used was a constant low-fat diet. Therefore, the objective of the present experiment was to test whether weight cycling on a constant high-fat or a medium-fat diet would also cause the same fatty acid metabolic changes with the selective depletion in LA and α-LnA. The previous study (Chapter 2) failed to demonstrate that "weight cycling induces obesity" when the WC rats were maintained on a low-fat diet (10% total energy). In this study, it was of interest to examine whether weight cycling would increase body fatness in the rats fed a constant high-fat diet (45% energy) and medium-fat diet (22% energy) after two consecutive WCs.

3.3 Materials and Methods

3.3.1 Animals and Diets

One hundred and forty male Sprague-Dawley rats were used in this study. They were housed with 2 rats per cage in an animal room at 23 °C with 12 h light/dark cycles. The rats were randomly divided into two groups: 75 rats for the high fat group (HF; 45% energy) and 65 rats for the medium-fat group (MF; 22% energy). The compositions of the two diets were shown in Table 3.1. Before WC, all the rats were allowed *ad libitum* access to their corresponding diet for 4 weeks. Their body weight and food intake were measured daily and the baseline food intake was determined. Corrections were made for spillage, which was minimized by placing the diet in a specially designed container (Figure 3.3).

After the 4 week stabilizing period, each group of the rats were further divided into *ad libitum* fed control group (CTL, 25 rats in HF group and 15 rats in MF group), and the weight cycled group (WC, 50 rats in both HF and MF groups). All the CTL rats were allowed free access to their diet and tap water throughout the period studied, while the WC rats were only allowed free access to tap water and they were subjected to two energy restriction-refeeding cycles.

The WC rats in both HF and MF group were subjected to 40% food restriction and the amount of food given during fasting was calculated according to the baseline food intake. In the HF group, two WCs were induced by partially fasting the rats (food intake=12.5 g/rat/day) for a period of 7 days, followed by *ad libitum* refeeding for 10 days. The two WCs in the MF group were similarly induced, but the amount of food

given to the rats was different (15.5 g/rat/day). Food efficiency was calculated as the ratio of weight gain (g) in a given period to the total food intake (g) in that period (Brownell, 1986; Reed, 1993).

Table 3.1 Composition of the HF and MF diets

Ingredients	**Percentage by Weight**	
	HF	**MF**
Corn starch	28.8	33.8
Unhydrogenated canola oil	25.0	10.0
Casein	23.5	23.5
Sucrose	14.3	24.3
Mineral mixture-AIN 76	3.5	3.5
Alpharel	3.2	3.2
Vitamin mixture-AIN 76A	1.0	1.0
Choline Bitartrate	0.4	0.4
DL-methionine	0.3	0.3
Total	100.0	100.0

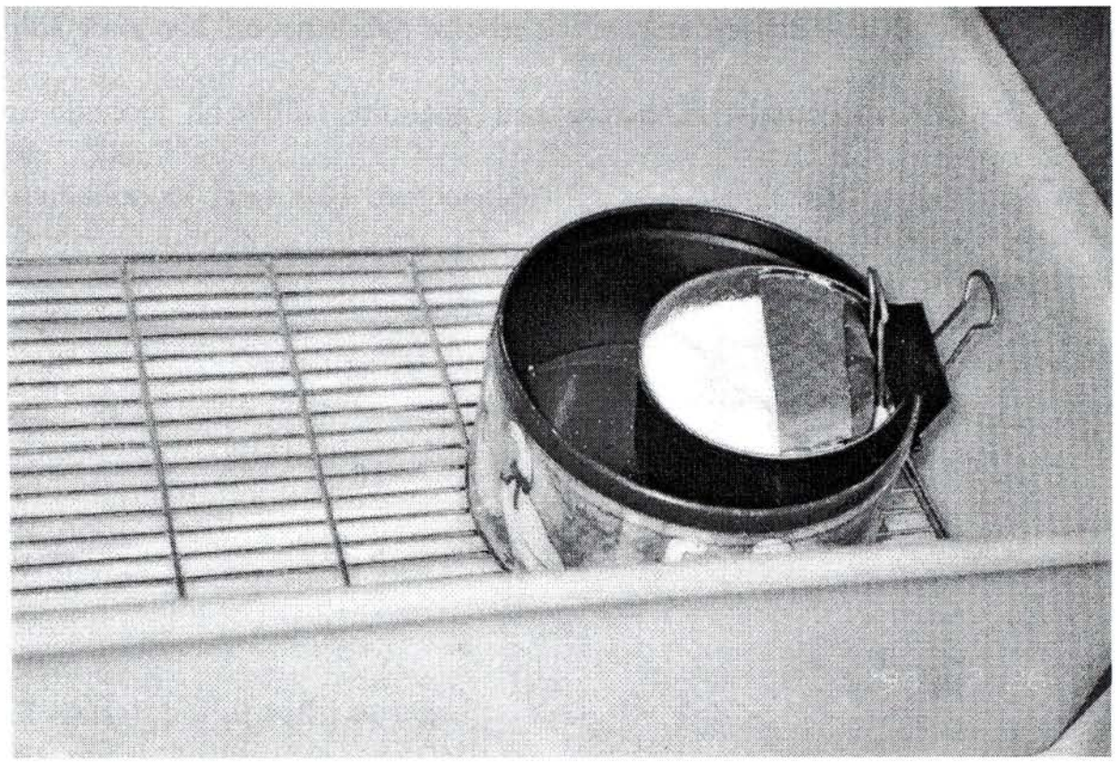


Figure 3.3 Diet Container.

At the beginning of each WC, rats from both HF and MF CTL groups were killed (Day 0), and the remaining CTL rats were killed at intervals: Day 7,17, 24 and 34 for HF, and Day 17 and 34 for MF. For the WC rats, they were killed at Day 1, 7, 8, 12, 17, 18, 24, 25, 29 and 34 (n=5).

All the rats were killed under nitrogen anesthesia and exanguinated via the abdominal aorta into a syringe. The serum was separated from the whole blood by centrifugation (2000 g for 15 min) and stored in aliquots at -76 °C. Liver, epididymal,

and perirenal adipose tissue were removed from the abdomen, blotted dry and then weighed. Except a portion of epididymal adipose which was used for adipocyte analysis, liver and the remaining adipose tissue were washed with chilled 0.9% saline, freeze-clamped in liquid nitrogen and stored in aliquots at -76 °C for the determination of fatty acid composition. Carcass [whole body - perirenal adipose tissue (2 pads) - epididymal adipose tissue (2 pads) - liver -blood] was also retained for lipid analysis. This study was approved by the Animal Care Committee of the Chinese University of Hong Kong.

3.3.2 Analysis of Adipocytes

The number and size of adipocytes were determined according to Cheung (1986) with slight modifications. Epididymal fat pads after the removal were immediately rinsed with 37 °C 0.9% saline. After dissecting free of blood vessels, the fat pads were minced. The minced adipose tissues (1.5g) were suspended in 4 % BSA-KRB (1 ml/g tissue) containing collagenase (1 mg/ml, from *Clostridium Histolytium*, Sigma Chemical, St. Louis, MO, USA) in 50 ml Falcon tubes. They were then incubated at 37 °C with continuous shaking (100 rpm) for 60 min.

Collagenase treatment was terminated by adding 2 volumes of 37 °C 1% BSA-KRB. Isolated adipocytes were collected by filtering the mixture through a layer of cheesecloth which was immersed in 1% BSA-KRB buffer in advance. The filtrate was allowed to stand for 1-3 min in a 37 °C incubator. After the adipocytes had floated onto the surface, the buffer was removed with a plastic Pasteur pipette. Adipocytes

were re-suspended in 3 ml of BSA-KRB and swirled gently to disperse the cells. Finally, 0.1 ml of adipocytes solution was pipetted onto the Haemacytometer. Adipocytes were taken and observed under a Zesis microscope with a proper magnification (50x). The number and diameter of isolated adipocytes were measured in the photographs. To avoid any bias in sampling, 4-5 photographs were prepared for each adipocyte sample. All the cells in a ruled area were counted. Since the released adipocytes from 1.5 g of adipose tissue were re-suspended in 3 ml of buffer, and the whole ruled area was 3 mm x 3 mm with a depth of 0.1 mm, the total volume of ruled area was $9x10^{-4}$ ml. Thus, the cell concentration is:

$$\frac{\text{cell number in the ruled area}}{(9x10^{-4})\,(1.5/3)} \quad \text{cells/g adipose tissue.}$$

The size of adipocytes was determined by counting the cell distributed around the central horizontal line in photographs. Assuming the adipocytes were spherical, the cell volumes could be calculated :

$$\text{cell volume} = (\pi/6)\,(3\sigma^2 + \bar{x}^2) \times \bar{x},$$

where $\bar{x}$ = mean cell diameter,

σ^2 = variance of the cell diameter (Goldrick, 1967).

3.3.3 Fatty Acid Analysis

Total lipids derived from adipose tissue, liver and carcass were extracted by homogenizing in chloroform-methanol (2:1, v/v). Heptadecanoic caid (Sigma), tri-heptadecanoic acid (Sigma) and L-α-phosphatidylcholine diheptadecanoyl (Sigma) were added as internal standards to quantitate the free fatty acids (FFA), triglycerides (TG) and phospholipids (PL), respectively. The chloroform-methanol phase

containing the lipid extracts was dried under a gentle stream of nitrogen and redissolved in chloroform, which was then applied to a thin-layer chomatography (TLC) plate (20x20 cm, precoated with 250μm silica gel 60 Å; Macherey-Naged, Duren, Germany) to separate different lipid classes. A solvent system of hexane : diethyl ether : acetic acid (80:20:10, v/v/v) was used for development. The bands containing FFA, TG and PL were scratched off the plate and the lipids extracted were converted to methyl esters by using a mixture of 14% BF_3 in methanol and toluene (v/v, 1:1) under nitrogen at 90 °C for 45 min. The fatty acid methyl esters in FFA, TG, PL and total lipids were analyzed by GLC as described in Section 2.3.2, Chapter 2.

3.3.4 Determination of Serum Cholesterol, Triglycerides and Glucose

Total serum cholesterol, serum triglycerides and serum glucose were determined by using the enzymatic kits (Sigma).

3.3.5 Statistics

Data were expressed as means ± SD. The analysis of fatty acid, adiopcytes, serum glucose, serum cholesterol and serum triglycerides were subjected to the analysis of variance (ANOVA) followed by the least significant difference test for statistical evaluation of the significant difference between the CTL and WC rats and only $p<0.05$ was considered statistically significant. This was done by running the data on personal computer ANOVA software (PC ANOVA for the IBM Personal Computer, Version 1.1; IBM, Armonk, NY, USA).

3.4 Results

3.4.1 Body Weight

Figures 3.4 and 3.5 showed the changes in body weight of the HF and MF groups over time. The body weight of all WC rats decreased gradually during the fasting periods of the two WCs, except on the first restriction day in the second cycle where the body weight of WC rats from the HF group dropped dramatically (Figure 3.4). At the beginning of refeeding, the body weight increased significantly, and then increased gradually until the end of the cycles. After two consecutive WCs, the final body weight of the WC rats was not significantly different from that of their corresponding controls.

The WC rats fed the high-fat diet had a weight reduction of 12.9 g and 22.4 g during the fasting periods of cycle 1 and cycle 2, respectively. During the refeeding period, the WC rats had a weight gain of 74.2 g and 74.8 g in cycle 1 and cycle 2, respectively (Figure 3.4). In contrast, the WC rats fed the medium-fat diet had a weight reduction of 31.4 g in cycle 1 and 20.2 g in cycle 2 during the food restriction days. The refeeding led to a total weight gain of 74.9 g and 64.4 g in cycle 1 and cycle 2, respectively (Fig 3.5).

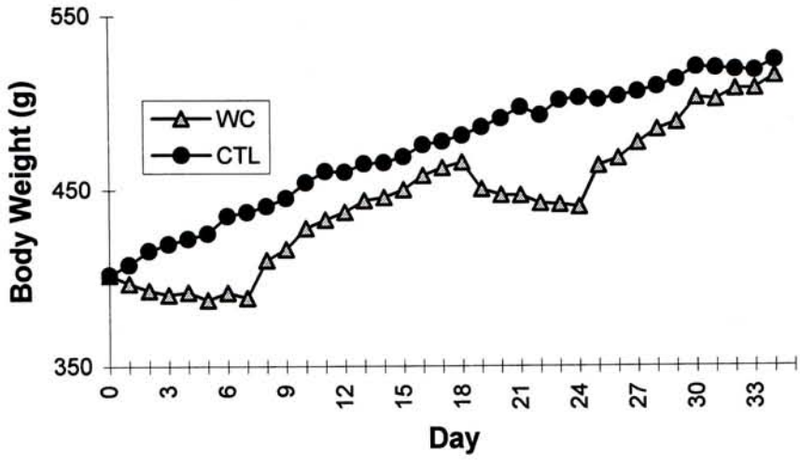


Figure 3.4 Body weight of rats fed HF-diet.

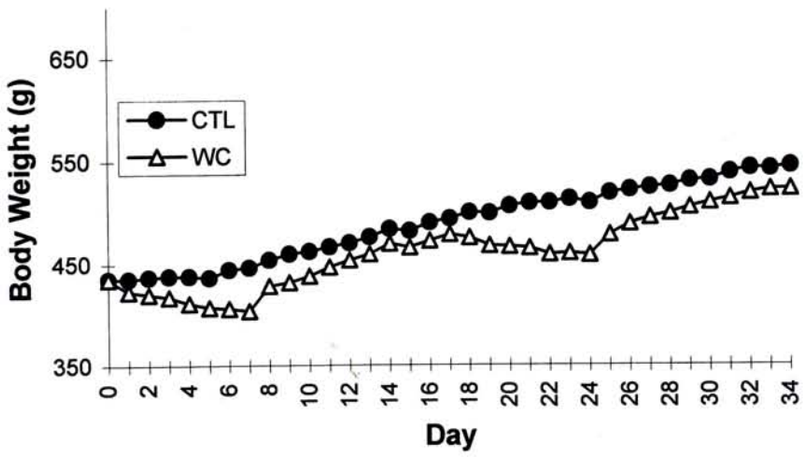


Figure 3.5 Body weight of rats fed MF-diet.

3.4.2 Food Intake and Food Efficiency

The time-course of food intake was graphically illustrated in Figure 3.6 and Figure 3.7. The food intake of the controls in HF and MF groups throughout the entire experiment was maintained at 20.9 ± 3.4 g/rat/day and 25.3 ± 2.8 g/rat/day, respectively. The weight reduction of WC rats was achieved by giving 40% food restriction for 7 days, i.e. 12.5 g/rat/day for HF group and 15.5 g/rat/day for MF group. Once the WC rats were allowed free access to the diet following food restriction, their food intake was gradually decreased and approached that of their CTL animals. However, during the first two refeeding days, their food intake was much higher than that of the CTL (Figure 3.6 and 3.7). Thereafter, their food intake gradually decreased and became similar to that of the CTL rats.

Table 3.2 showed the calculated food efficiency for all groups. There was no significant difference of food efficiency in overall 2 WCs (Days 1 - 34) between the WC and CTL rats in both HF and MF group. However, the food efficiency during WC 2 (Days 18 - 34) was significantly higher in WC rats than in CTL from the HF group. During refeeding periods (Days 8 - 17 and Days 25 - 34), the food efficiency was 1.72- and 3.26-fold higher in WC rats fed with HF diet compared with their CTL. In MF group, the food efficiency among WC rats was also 1.43- and 1.73-fold higher than that of their CTL during the first and second refeeding periods, respectively. However, the food efficiency of WC rats was similar in WC 1 and WC 2, first refeeding and second refeeding if the comparison was made within the diet with the same level of fat.

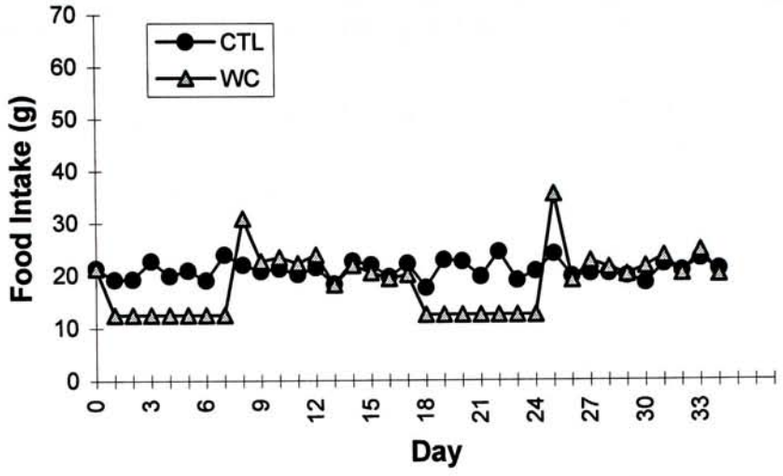


Figure 3.6 Food intake of rats fed HF-diet.

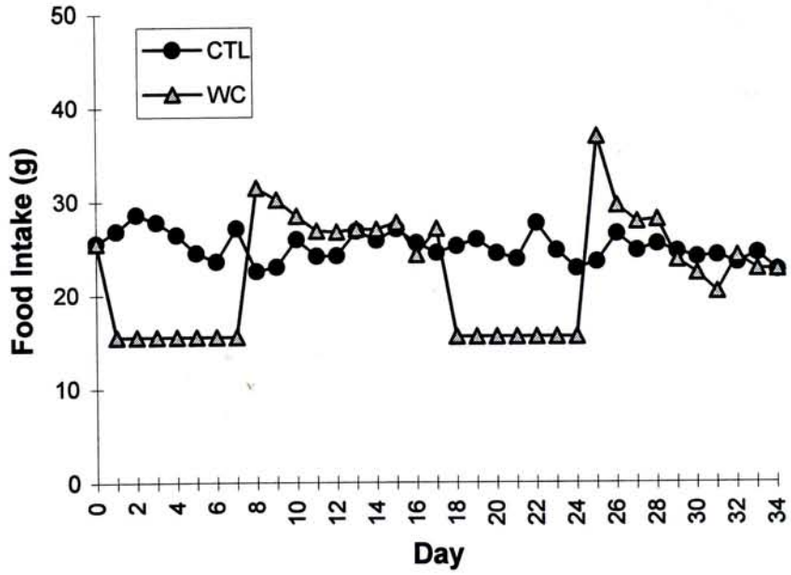


Figure 3.7 Food intake of rats fed MF-diet.

Table 3.2 Food efficiency of the rats in HF and MF groups

Period of Time	HF		MF	
	CTL	WC	CTL	WC
2 WCs (Days1-34)	17.21 ± 1.55	18.14 ± 1.45	12.90 ± 1.23	11.67 ± 0.82
WC 1 (Days 1-17)	21.55± 1.51	19.76 ± 0.99	13.54 ± 1.17	11.30 ± 0.68
WC 2 (Days 18-34)	12.89 ± 0.91	16.54 ± 1.78**	12.23 ± 1.34	12.06 ± 0.63
1st Food Restriction (Days 1-7)	24.69 ± 1.98	-	6.22 ± 0.94	-
1st Refeeding (Days 8-17)	19.38 ± 1.36	33.24 ±1.72***	18.97 ± 1.31	27.08 ± 1.42***
2nd Food Restriction (Days19-24)	16.95 ± 1.37	-	9.12 ± 0.64	-
2nd Refeeding (Days 15-34)	10.03 ± 0.57	32.66 ±1.28***	14.47 ± 1.25	24.97 ± 1.58***

Data are expressed as mean ± SD (n=5).
**p<0.01 and ***p<0.001, between the CTL and the corresponding WC rats.

3.4.3 Weight of Liver

Both HF and MF groups showed a similar pattern of change in liver weight (Figure 3.8 and Figure 3.9). During food restriction, the weight of liver in WC rats dropped dramatically. The decrease was more pronounced in the first food restriction days especially for the WC rats fed MF diet. During the first few refeeding days, their liver weight increased immediately and then gradually to reach the level of their corresponding CTL.

3.4.4 Weight of Adipose Tissue

The weight of epididymal and perirenal adipose fat pads changed similarly during weight cycling in both HF and MF groups (Figures 3.10, 3.11, 3.12 and 3.13). Although the weight of the two fat pads of WC rats decreased and increased concurrently with the change of body weight in the two cycles in both groups, the WC rats responded more prominently in the HF group during the refeeding periods when compared with the WC rats in MF group. In HF group, the weight of fat pads increased drastically especially in the first few refeeding days. Finally, the WC rats had a significantly higher fat pads weight than that of the CTL at the end of the cycle. In MF group, the fat pads of WC rats increased less pronouncedly during refeeding and their final weight was slightly lower than that of their CTL.

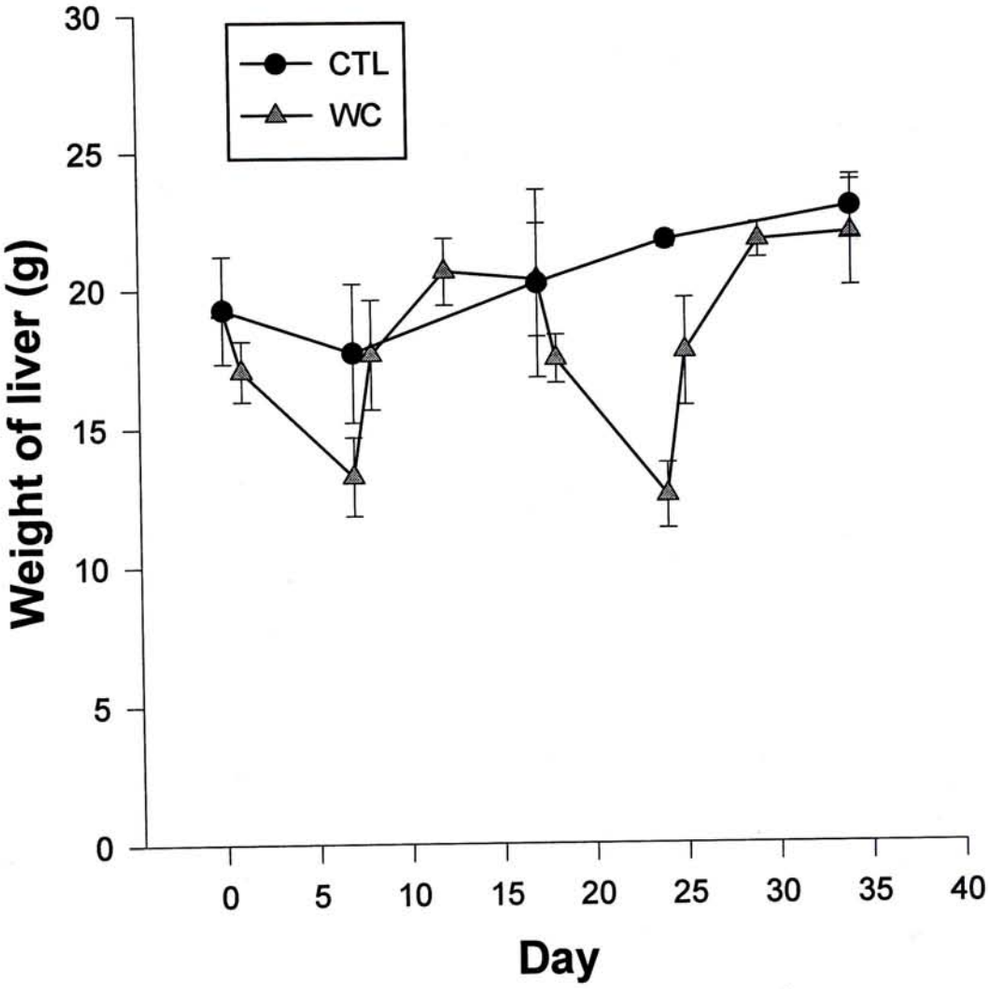


Fig 3.8 Weight of livers of rats fed HF-Diet.

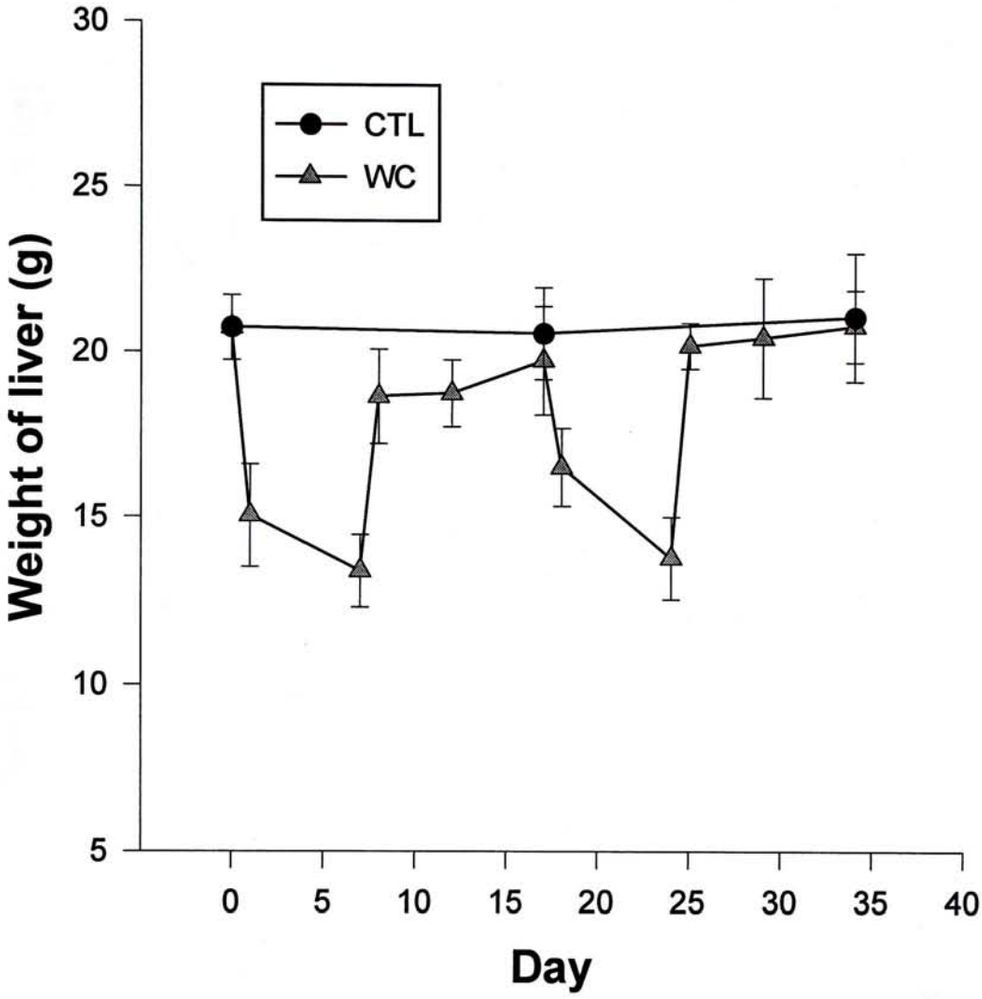


Figure 3.9 Weight of livers of rats fed MF-diet.

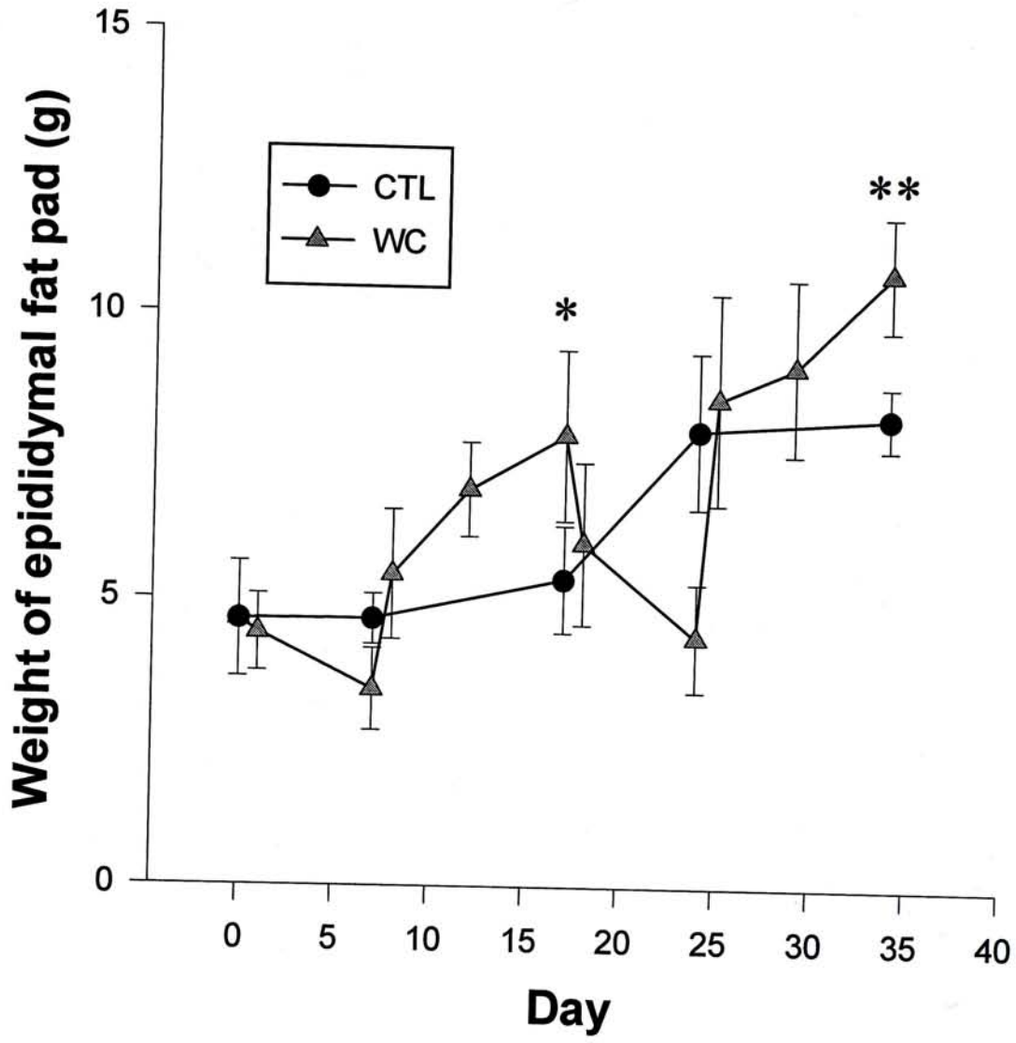


Figure 3.10 Weight of epididymal fat pads of rats fed HF-diet. (*p<0.05 and **p<0.01, difference between CTL and WC rats).

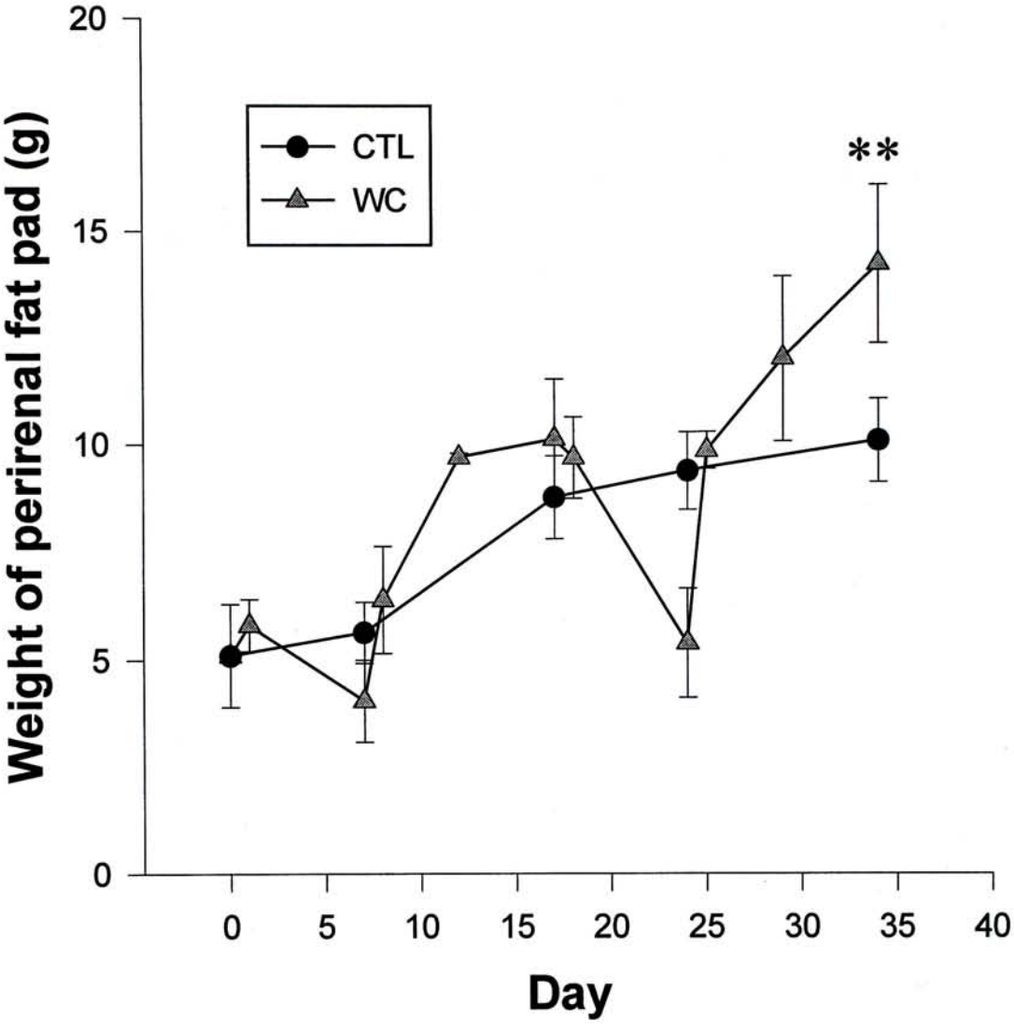


Figure 3.11 Weight of perirenal fat pads of rats fed HF-diet. (**p<0.01, difference between CTL and WC rats).

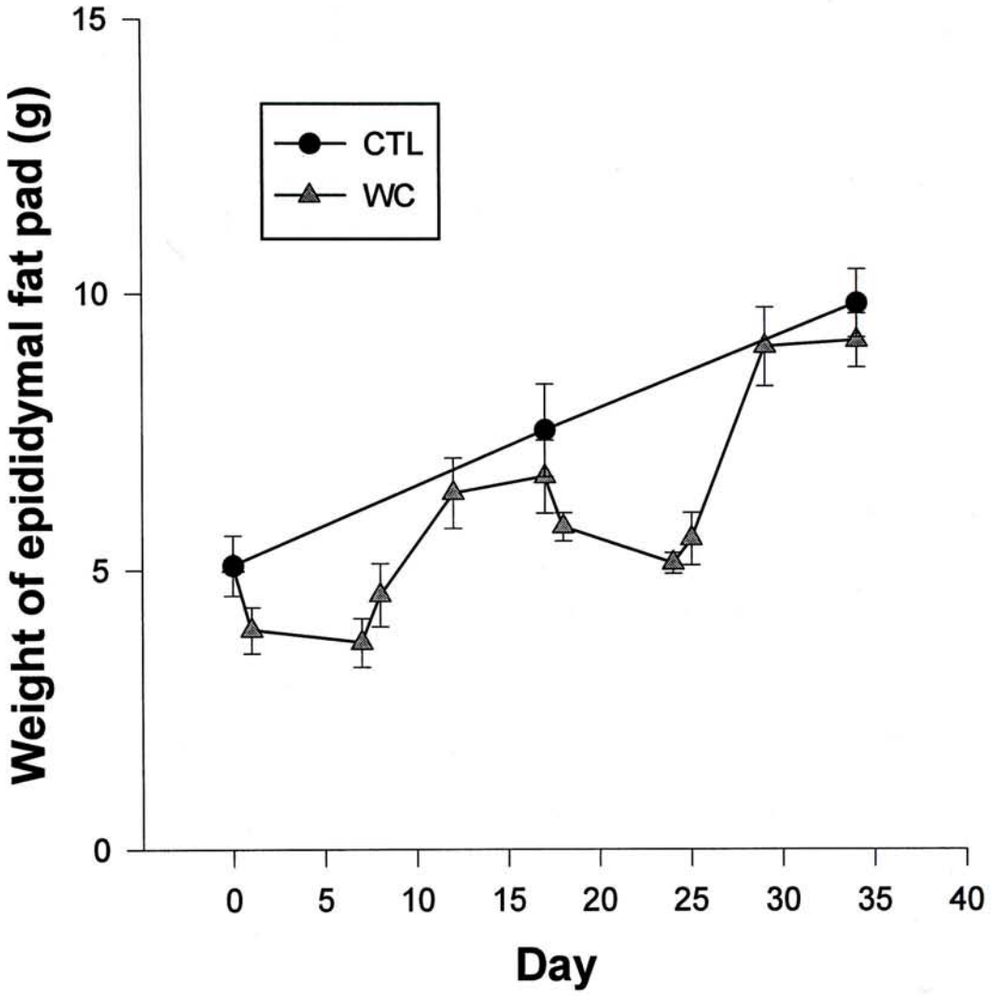


Figure 3.12 Weight of epididymal fat pads of rats fed MF-diet.

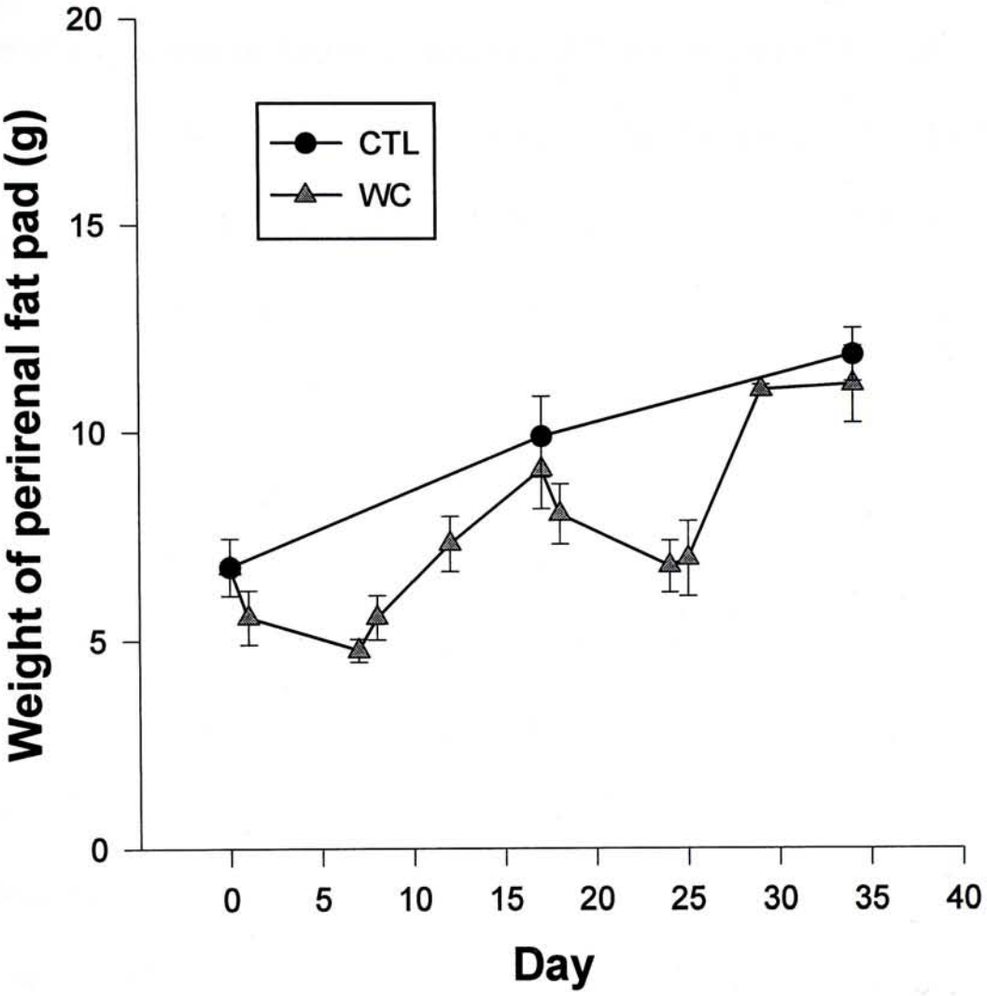


Figure 3.13 Weight of perirenal fat pads of rats fed MF-diet.

3.4.5 Number and Size of Adipocytes

After two WCs, the WC rats from both HF and MF groups had a similar number of adipocytes as their corresponding CTL rats (Figures 3.14 and 3.15). In the HF group, the number or density of adipocytes in the WC rats was $162.8 \pm 39.8 \times 10^4$ cells/g adipose tissue while that in the CTL was $170.2 \pm 35.6 \times 10^4$ cells/g adipose tissue. In the MF group, the number of adipocytes in the WC rats was $164.6 \pm 32.0 \times 10^4$ cells/g adipose tissue while that in the CTL rats was $169.6 \pm 32.8 \times 10^4$ cells/g adipose tissue.

Regarding the size of adipocytes, WC rats in HF group responded differently to weight cycling when compared with the WC rats in MF group (Figures 3.14 and 3.16). In HF group, the size of adipocytes of WC rats was considerably enlarged and was about 80% larger than that of the CTL ($p<0.01$). After two WCs, the average size of adipocytes of WC rats was $677.5 \pm 247.6 \times 10^3$ μm^3 while that in the CTL rats was $376.2 \pm 88.9 \times 10^3$ μm^3. In MF group, the average size of adipocytes in the WC rats was $366.5 \pm 159.4 \times 10^3$ μm^3 while that in the CTL was $377.3 \pm 77.1 \times 10^3$ μm^3, and there was no significant difference between WC rats and CTL rats.

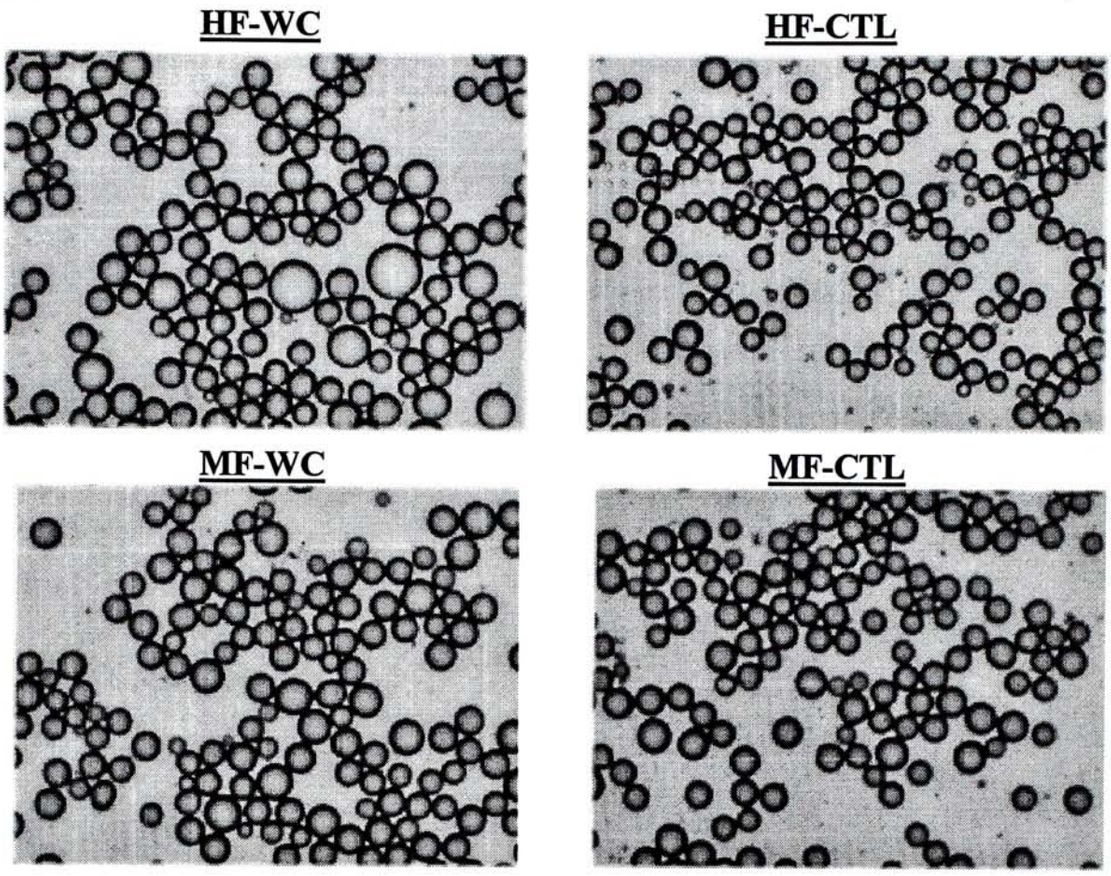


Figure 3.14 Typical samples of adipocytes.

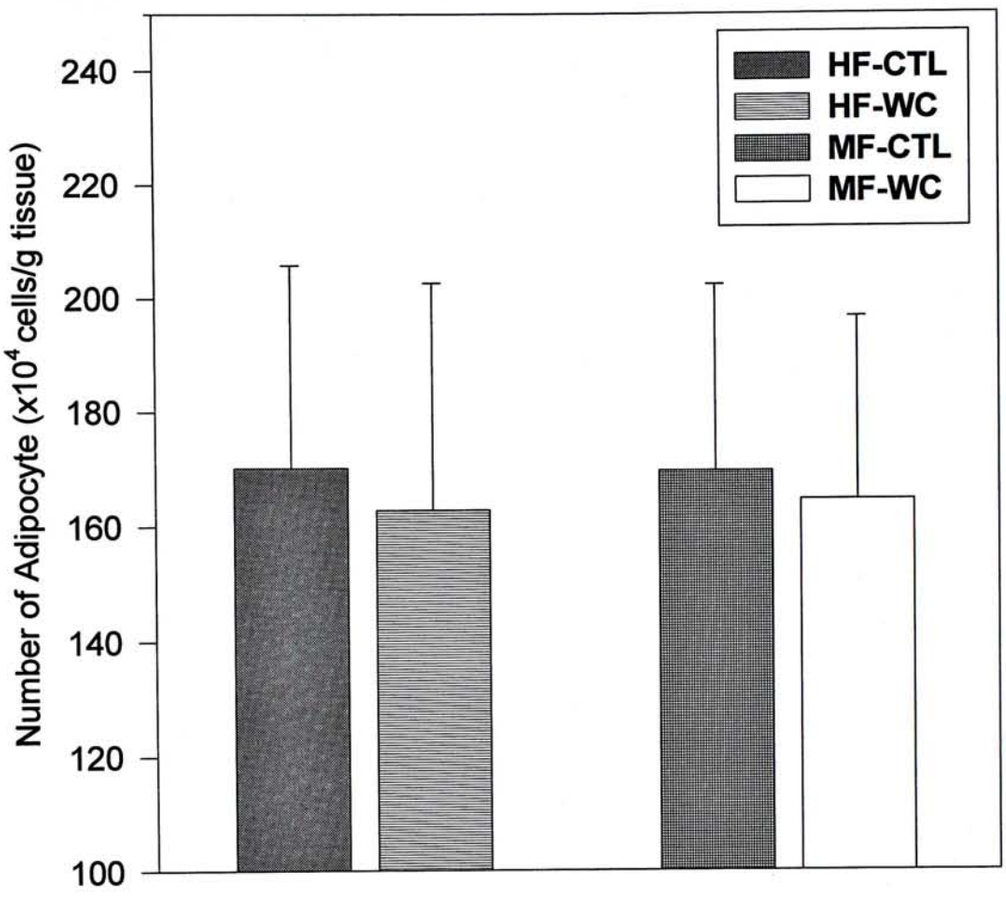


Figure 3.15 Effect of 2 WCs on the number of adipocytes of rats fed HF diet or MF-diet.

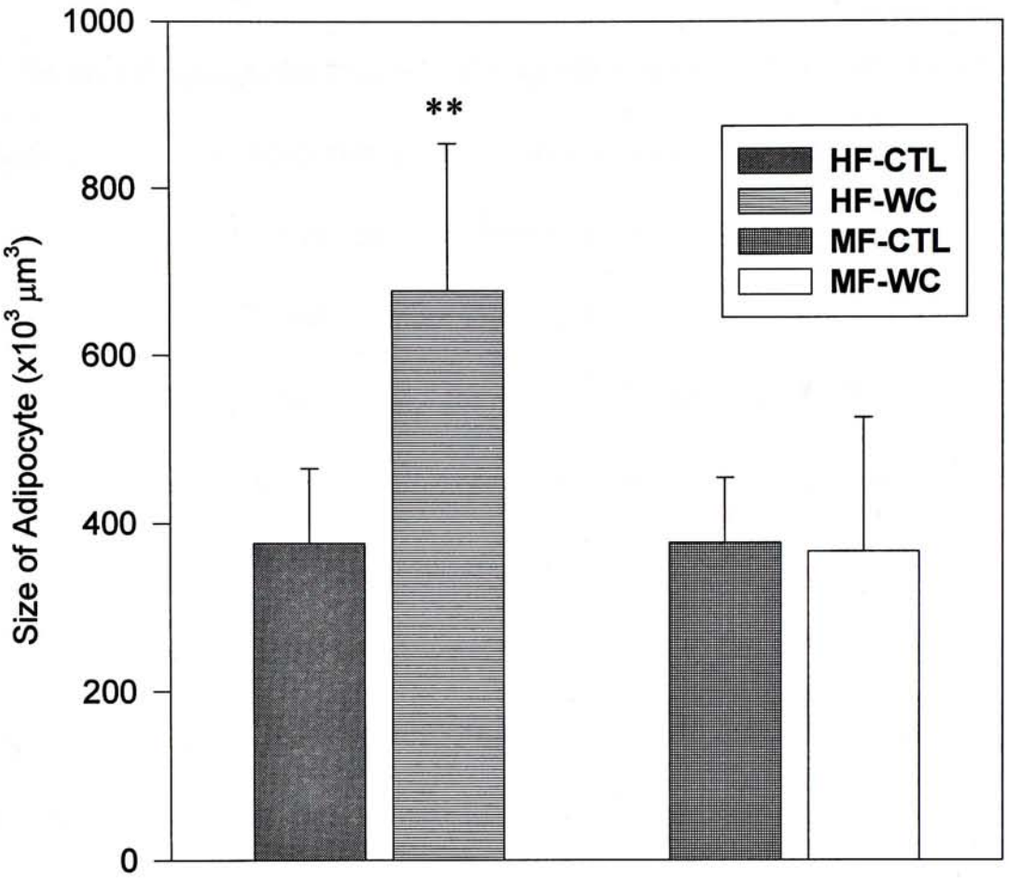


Figure 3.16 Effect of 2 WCs on the size of adipocytes of rats fed HF diet or MF-diet. (**p<0.01, significant difference between CTL and WC rats.)

3.4.6 Serum Triglycerides, Cholesterol and Glucose

3.4.6.1 Serum Triglycerides

In the HF group, the serum total triglycerides of the WC rats decreased during the food restriction in both two cycles, and then rose to a significantly higher level than that of the CTL rats during refeeding (Figure 3.17). However, the triglycerides concentration of the WC rats was not significantly different from that of the CTL rats at the end of WC 2. Moreover, for both WC rats and CTL rats, their serum triglycerides levels at the end of the experiment were higher than that at the beginning.

In the MF group, the serum total triglycerides of the WC rats also fluctuated in a pattern similar to that in HF group (Figure 3.18). However, the final concentration of the triglycerides of all rats remained the same as that at the beginning of the experiment.

3.4.6.2 Serum Cholesterol

Similar fluctuation of serum cholesterol concentration was observed in the WC rats fed HF diet during weight cycling (Figure 3.19). Besides, there was also no significant difference between the WC and CTL rats at the end of cycle 2. In MF group, though the WC rats had a much higher serum cholesterol level than their CTL during the refeeding, there was no difference between the two groups at the end of the experiment (Figure 3.20).

3.4.6.3 Serum Glucose

In HF group, the serum glucose concentration showed no difference between the WC and CTL rats (Figure 3.21). In MF group, the serum glucose concentration in WC rats decreased to a greater extent than that of HF group during the food restrictions (Figure 3.22). The final serum glucose level in WC rats was, however, slightly higher than that of the CTL rats at the end of cycle 1 and cycle 2.

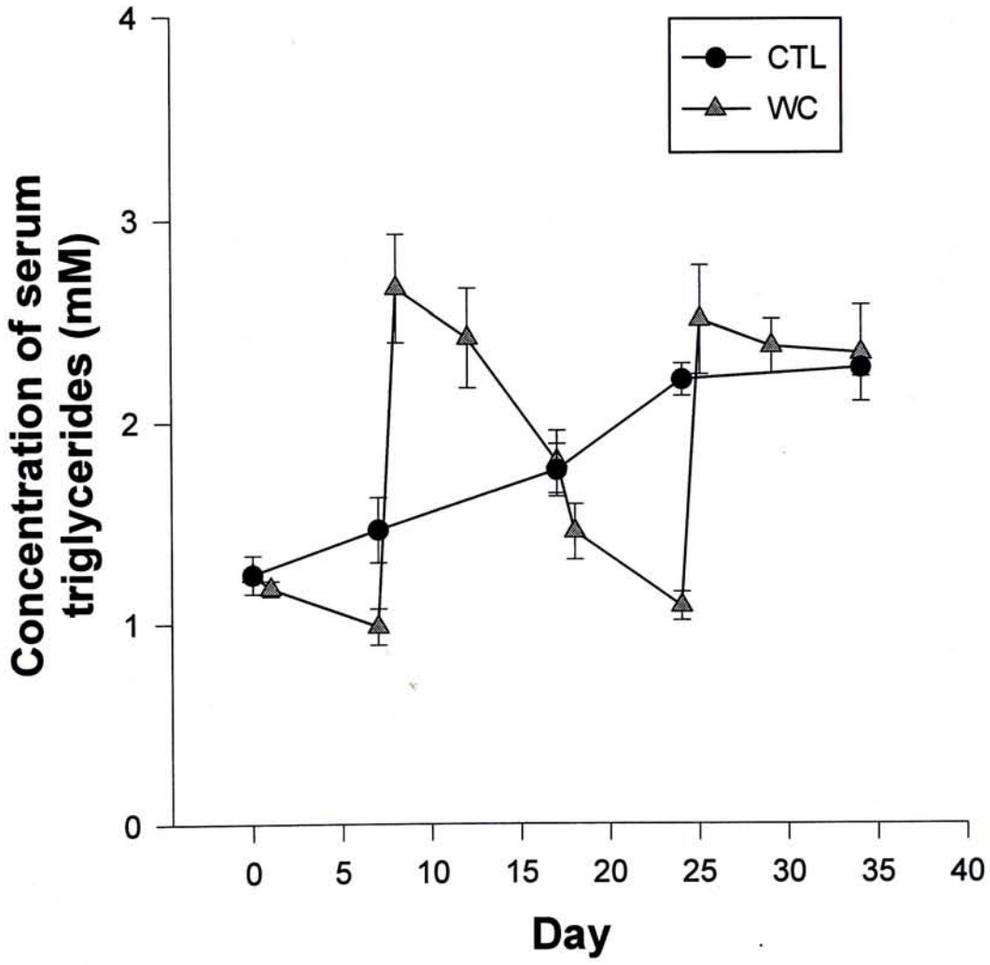


Figure 3.17 Concentration of serum triglycerides of rats fed HF-diet.

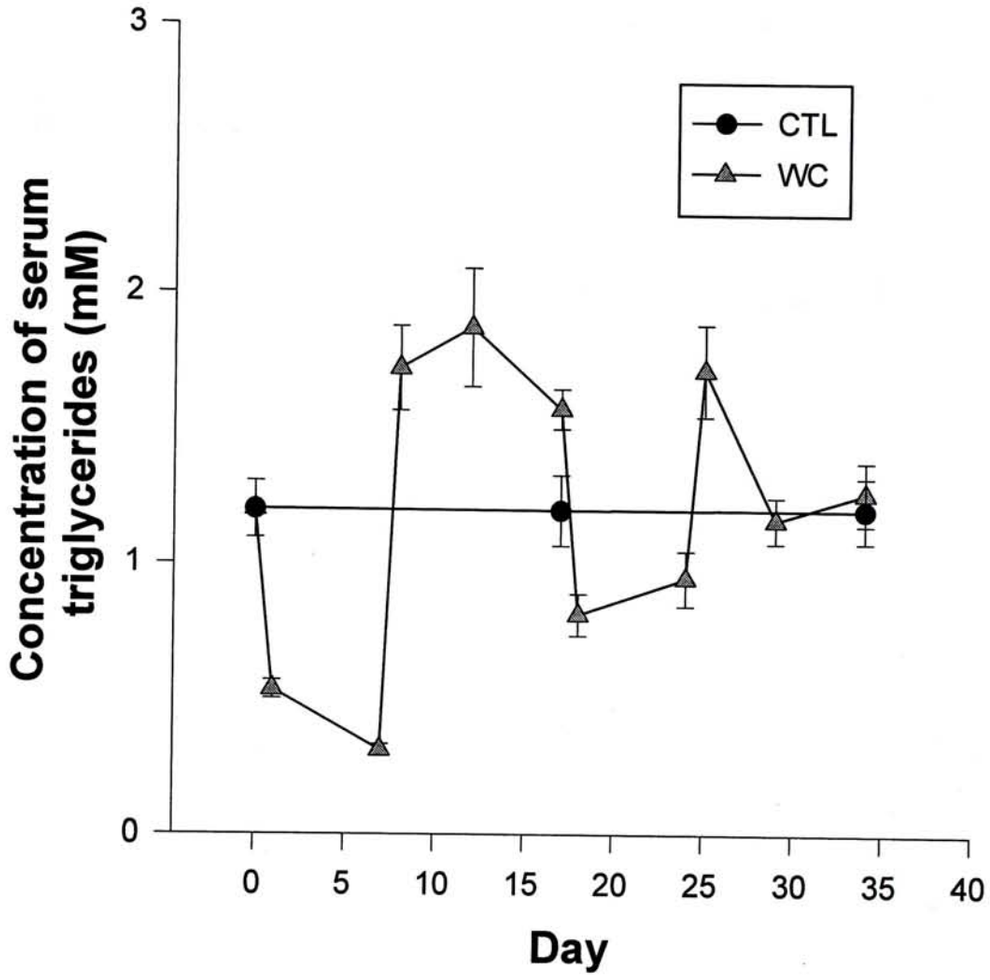


Figure 3.18 Concentration of serum triglycerides of rats fed MF-diet.

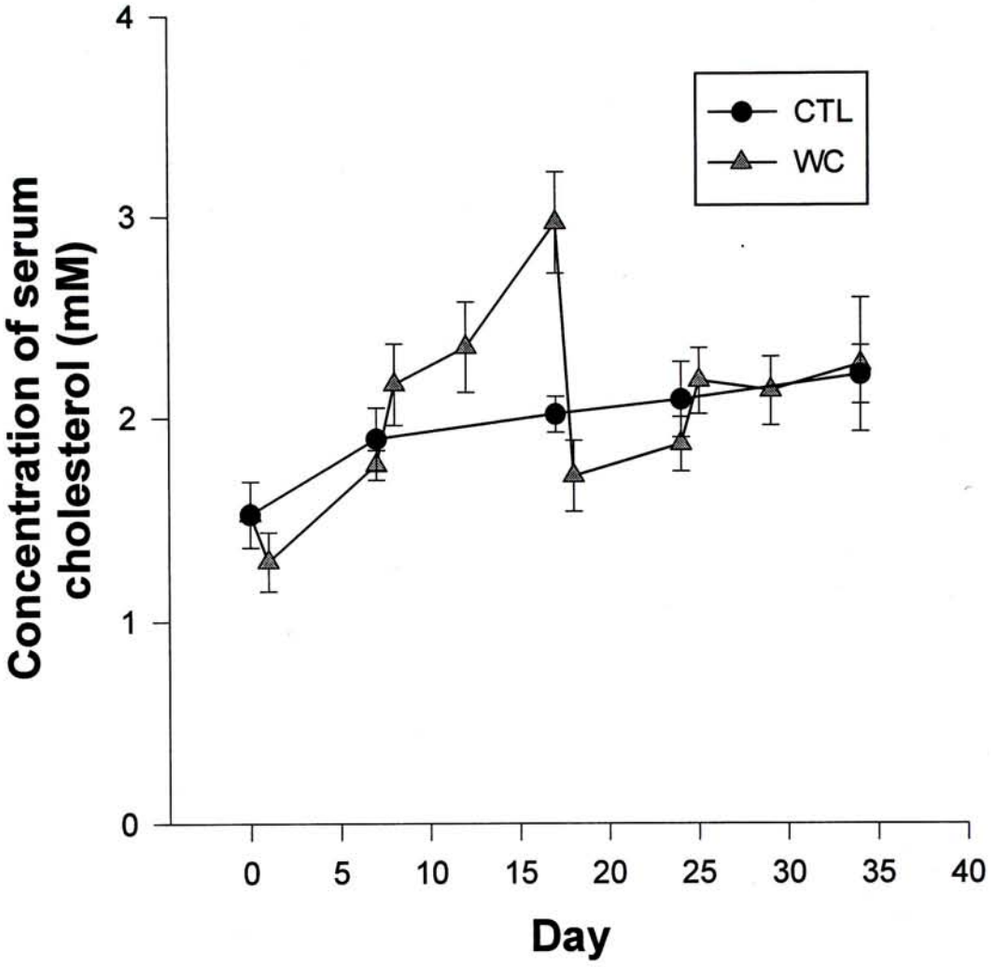


Figure 3.19 Concentration of serum cholesterol of rats fed HF-diet.

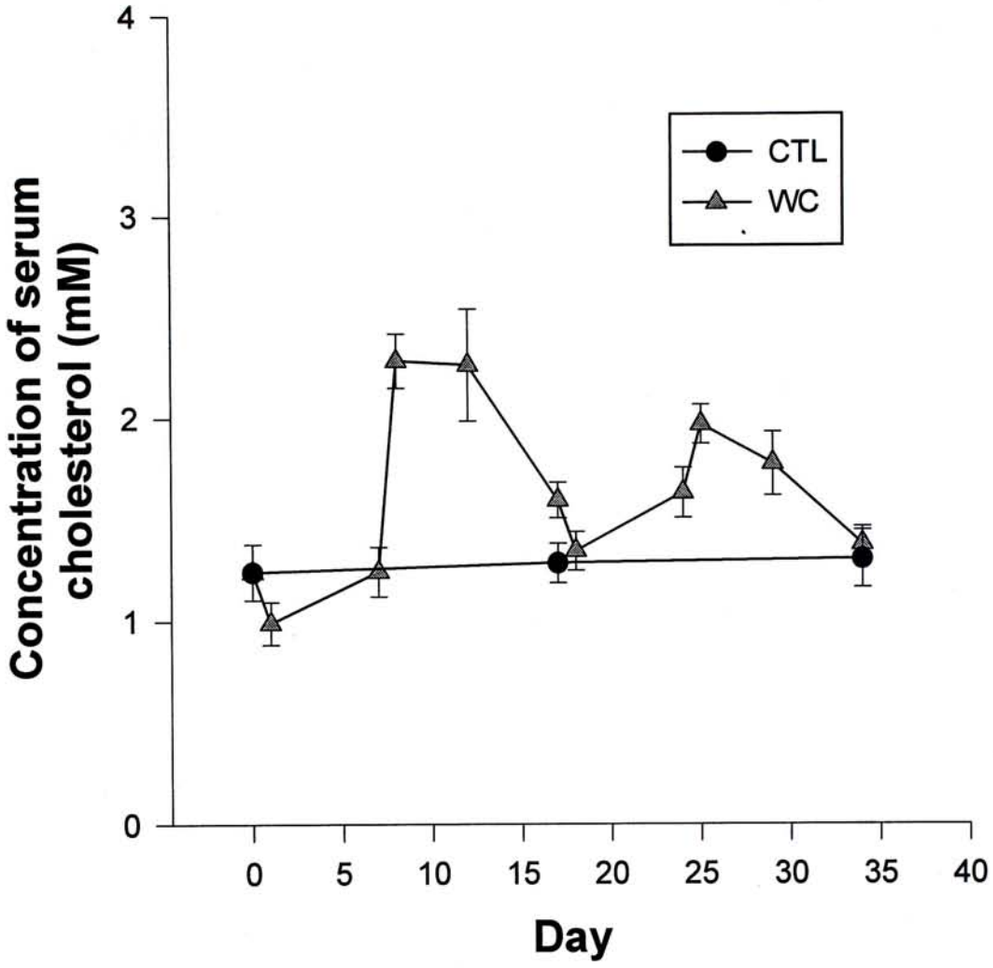


Figure 3.20 Concentration of serum cholesterol of rats fed MF-diet.

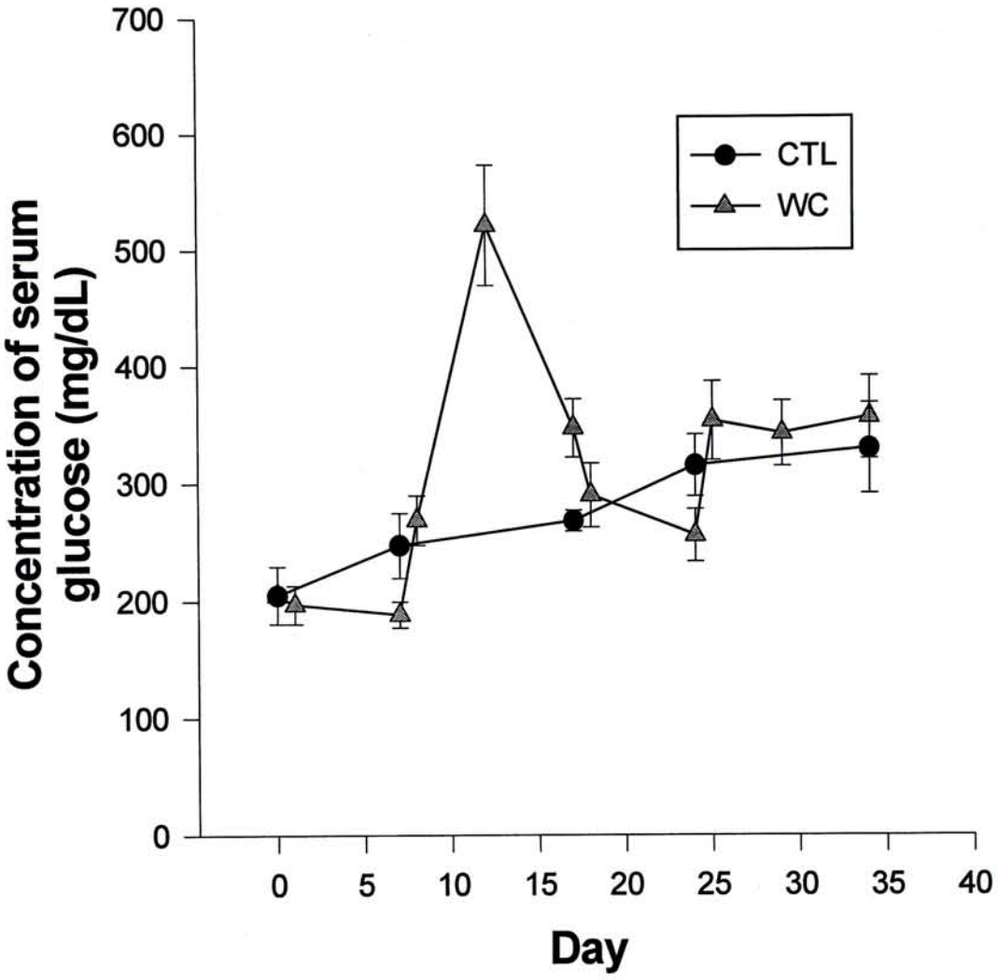


Figure 3.21 Concentration of serum glucose of rats fed HF-diet.

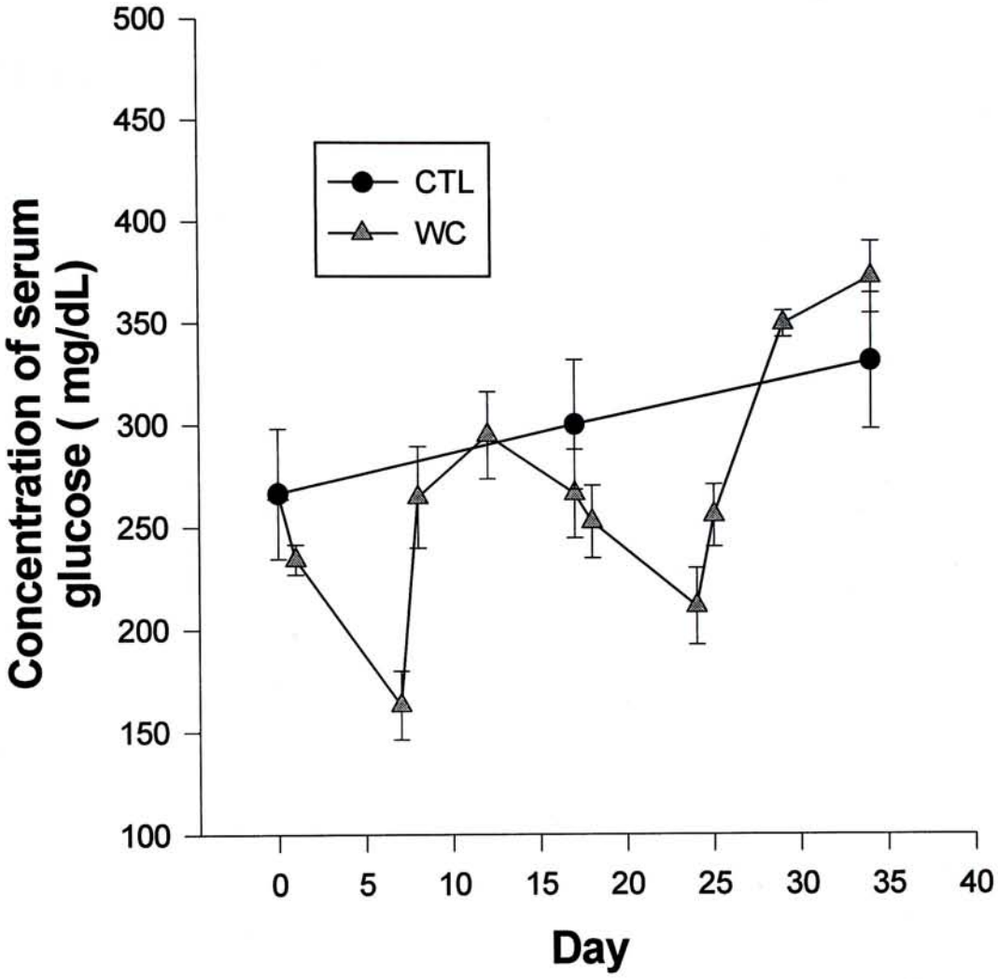


Figure 3.22 Concentration of serum glucose of rats fed MF-diet.

3.4.7 Fatty Acid Composition

3.4.7.1 Carcass Fatty Acids

3.4.7.1.1 Carcass Total Lipids (TL)

The effects of two WCs on carcass total fatty acid composition in HF and MF groups were shown in Figures 3.23, 3.24, 3.25 and 3.26. In HF group, the percentages of 18:2n-6 and 18:3n-3 gradually decreased in WC rats during the two food restriction periods. In contrast, 16:0, 16:1n-7 and 18:0 were significantly increased in WC rats.

In MF group, 18:2n-6 and 18:3n-3 were similarly reduced in WC rats while 16:0, 16:1n-7 and 18:0 were significantly higher in WC rats compared with the control group. The proportion of 14:0 in WC rats was also markedly increased in WC rats at the end of the second cycle.

3.4.7.1.2 Carcass Phospholipids (PL)

Throughout the two WCs, the fatty acid composition of carcass phospholipids was nearly constant in all rats and the fatty acid composition in WC rats was not significantly different from that in the CTL rats (Tables 3.3 and 3.4).

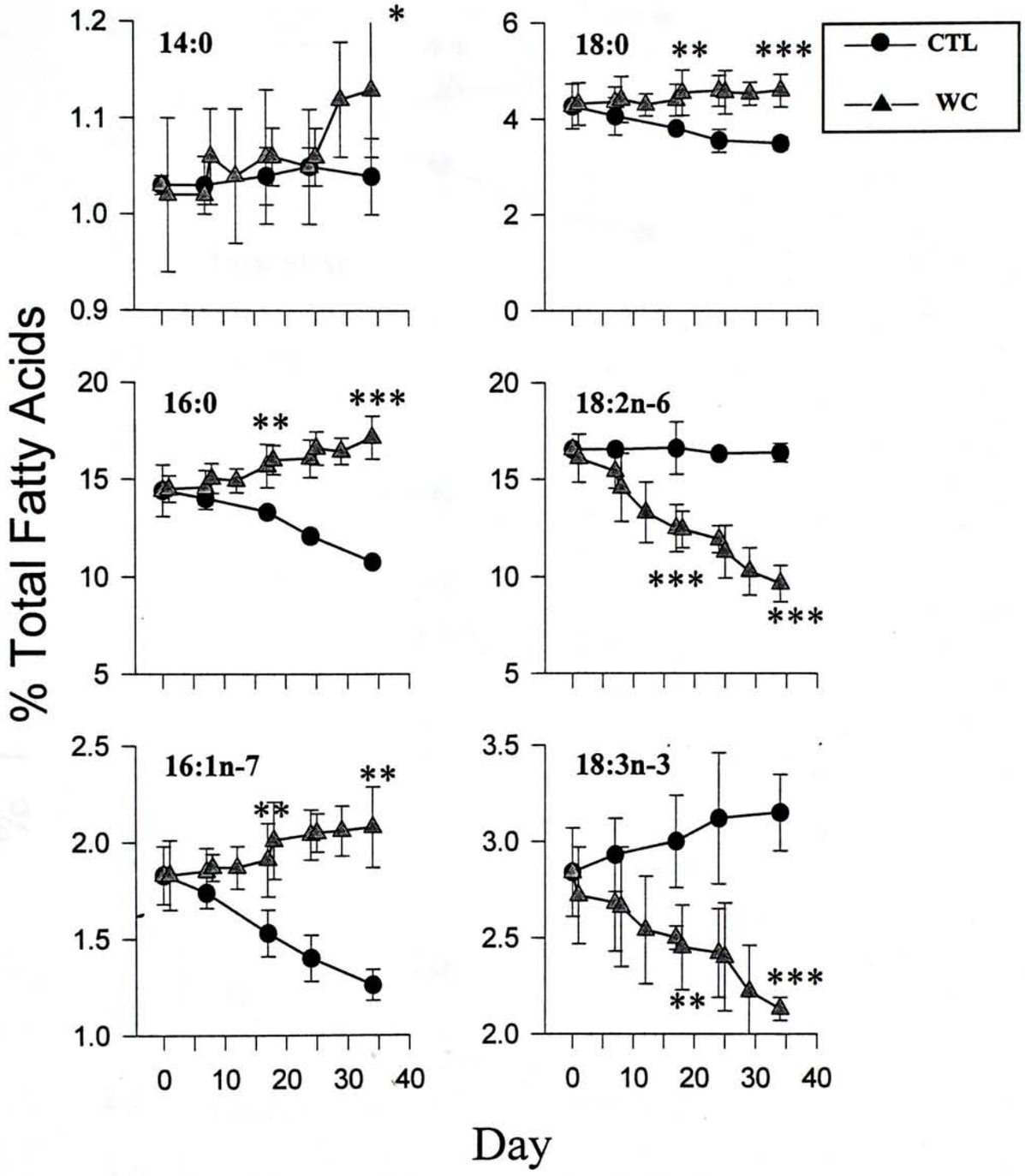


Figure 3.23 Time-course changes in individual fatty acid in carcass TL in rats fed HF diet. (*p<0.05, **p<0.01 and ***p<0.001, difference between WC and CTL rats.)

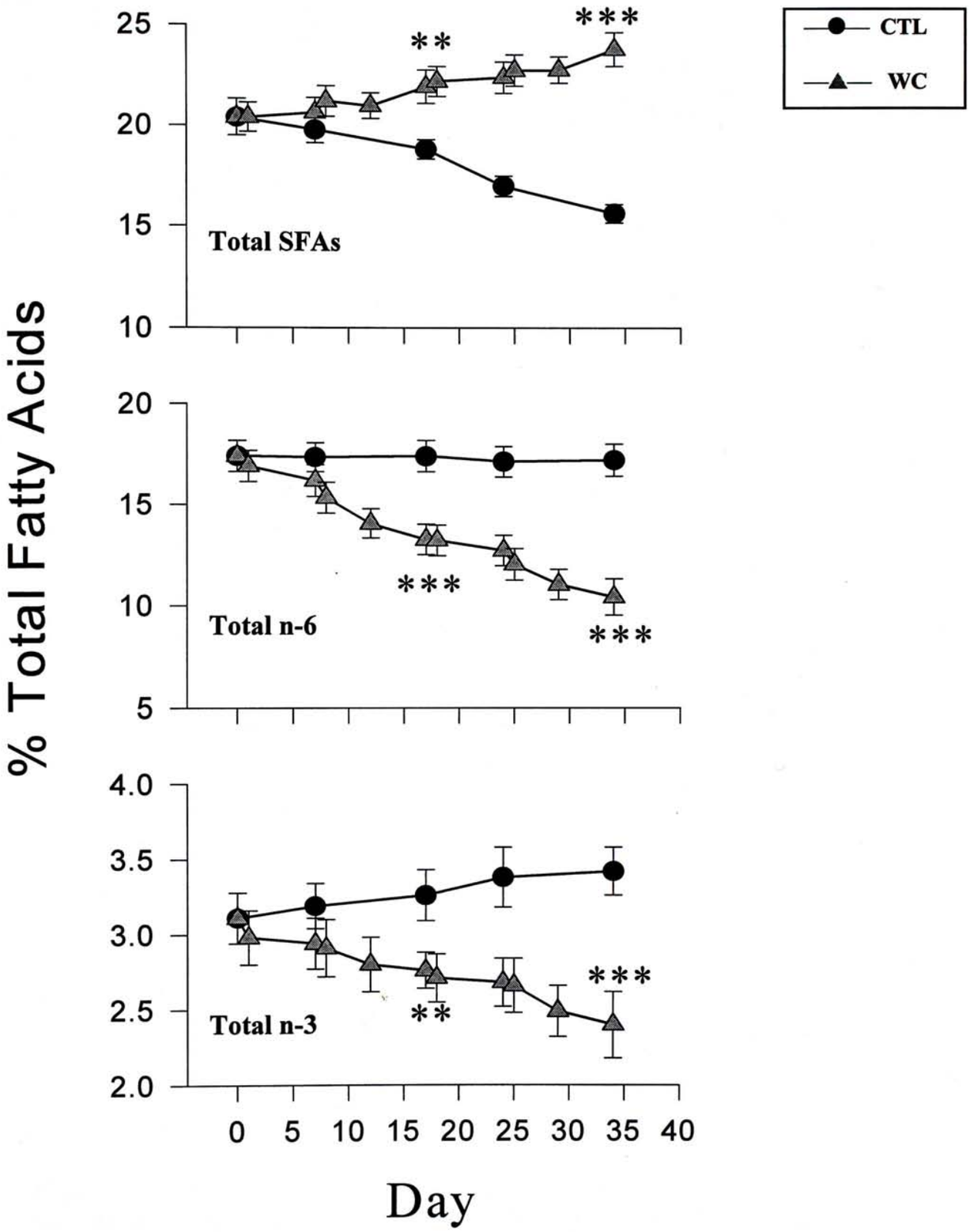


Figure 3.24 Time-course changes in total SFAs, total n-6 and total n-3 in carcass TL in rats fed HF diet. (**p<0.01 and ***p<0.001, difference between WC and CTL rats.)

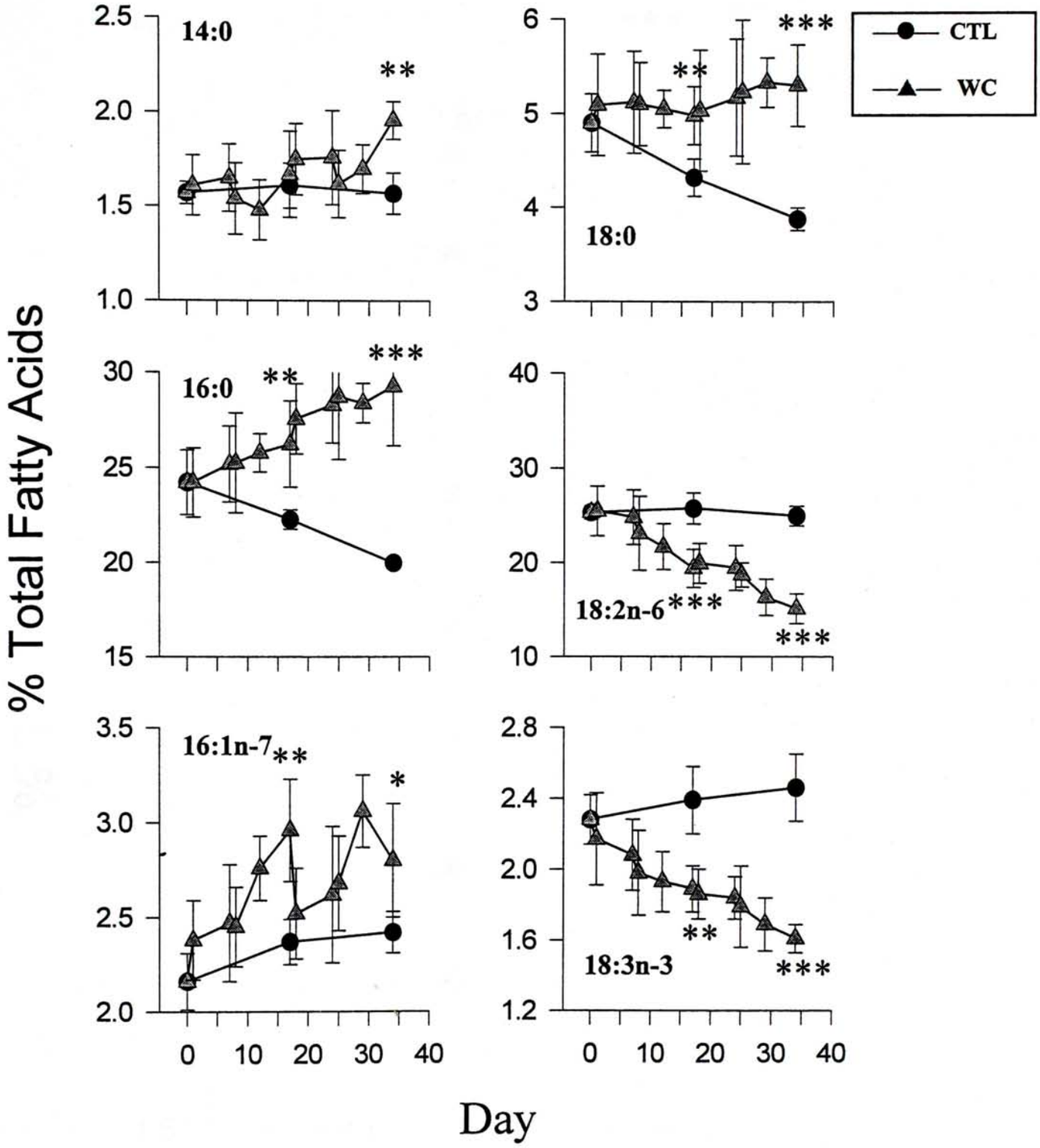


Figure 3.25 Time-course changes in individual fatty acid in carcass TL in rats fed MF diet. (*p<0.05, **p<0.01 and ***p<0.001, difference between WC and CTL rats.)

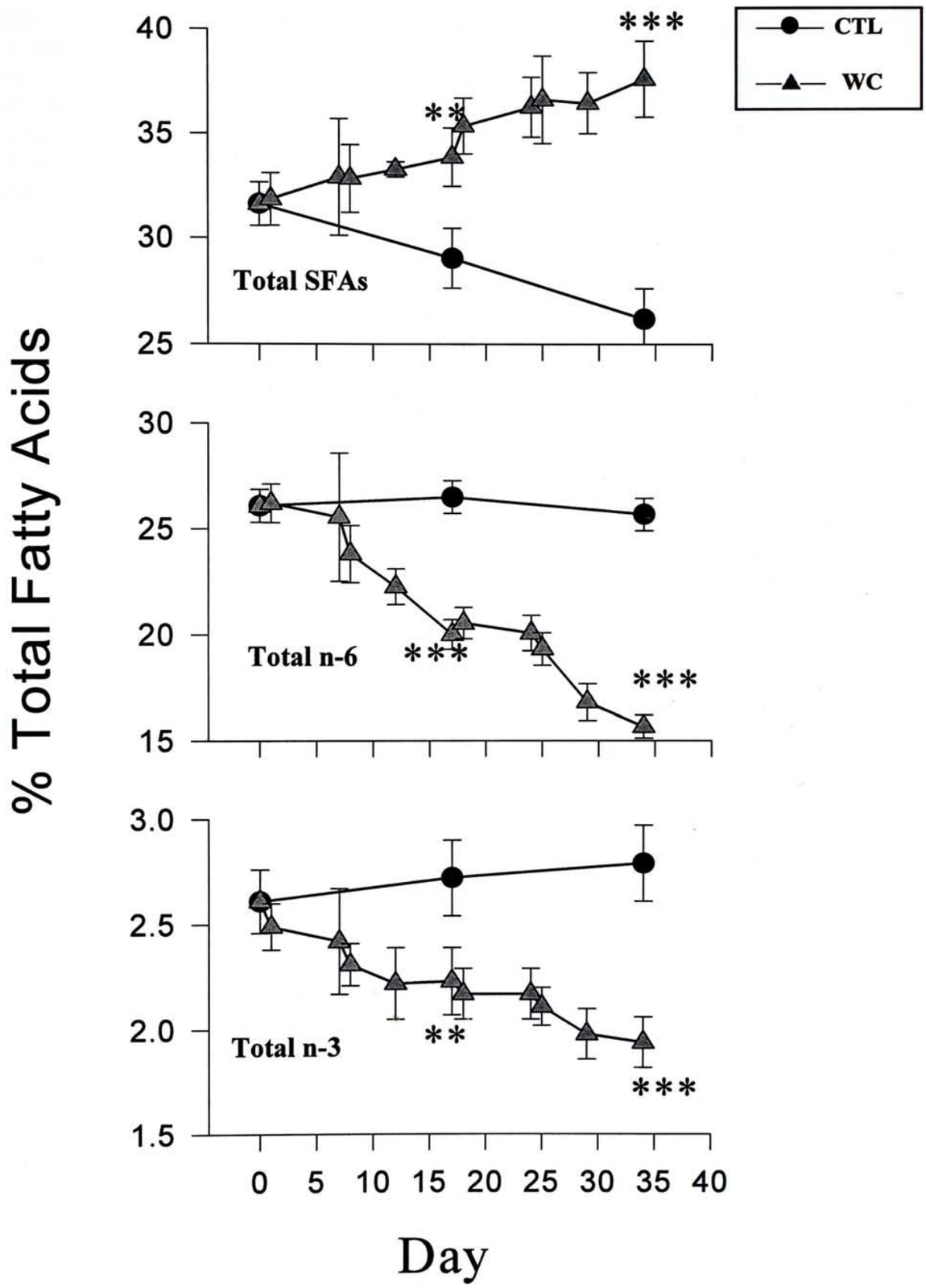


Figure 3.26 Time-course changes in total SFAs, total n-6 and total n-3 in carcass TL in rats fed MF diet. (**p<0.01 and ***p<0.001, difference between WC and CTL rats.)

Table 3.3 Fatty Acid Composition of carcass PL in rats of HF group

Fatty Acids	**CTL**				
	Day 0	**Day 7**	**Day 17**	**Day 24**	**Day 34**
14:0	0.43 ± 0.10	0.45 ± 0.07	0.43 ± 0.13	0.43 ± 0.07	0.43 ± 0.06
16:0	11.00 ± 1.64	10.92 ± 1.52	10.22 ± 1.36	10.59 ± 1.35	10.75 ± 1.23
16:1n-7	0.42 ± 0.09	0.45 ± 0.07	0.46 ± 0.08	0.46 ± 0.07	0.48 ± 0.11
18:0	6.89 ± 1.41	6.45 ± 1.07	6.53 ± 1.16	6.57 ± 1.39	6.52 ± 1.23
18:1n-9	36.17 ± 2.61	38.49 ± 3.24	39.21 ± 3.58	39.60 ± 5.40	37.43 ± 3.05
18:1n-7	3.34 ± 0.20	3.72 ± 0.37	3.84 ± 0.41	3.63 ± 0.22	3.86 ± 0.43
18:2n-6	19.78 ± 2.53	19.43 ± 2.76	19.61 ± 1.19	18.72 ± 2.43	18.55 ± 3.51
20:3n-6	0.31 ± 0.08	0.26 ± 0.07	0.27 ± 0.09	0.33 ± 0.04	0.36 ± 0.06
20:4n-6	4.48 ± 0.79	4.56 ± 1.30	4.41 ± 1.15	4.31 ± 0.73	4.67 ± 0.43
22:4n-6	0.91 ± 0.23	1.06 ± 0.20	0.82 ± 0.22	0.95 ± 0.10	0.91 ± 0.09
22:5n-6	0.19 ± 0.05	0.23 ± 0.04	0.18 ± 0.03	0.15 ± 0.05	0.17 ± 0.04
18:3n-3	2.25 ± 0.30	2.11 ± 0.30	2.49 ± 0.34	2.69 ± 0.55	2.41 ± 0.33
20:5n-3	0.77 ± 0.07	0.65 ± 0.10	0.67 ± 0.28	0.70 ± 0.17	0.79 ± 0.12
22:5n-3	0.77 ± 0.21	0.63 ± 0.15	0.73 ± 0.38	0.71 ± 0.23	0.82 ± 0.12
22:6n-3	4.23 ± 0.76	4.33 ± 0.92	4.60 ± 1.27	4.16 ± 0.91	4.92 ± 0.89
Total SFAs	18.65 ± 1.87	18.14 ± 2.58	17.51 ± 1.75	18.00 ± 1.95	18.05 ± 2.17
Total MUFAs	40.01 ± 2.52	42.82 ± ± 3.26	43.67 ± 3.56	44.12 ± 5.55	42.02 ± 2.89
Total n-6	25.69 ± 2.33	25.54 ± 2.25	25.28 ± 1.77	24.46 ± 2.32	24.66 ± 3.49
Total n-3	8.02 ± 1.01	7.73 ± 1.01	8.49 ± 2.18	8.26 ± 0.51	8.94 ± 0.97
Total FAs (mg/g)	26.14 ± 3.07	26.28 ± 3.42	26.43 ± 3.01	24.77 ± 5.77	25.60 ± 3.57

Data are expressed as mean ± SD (n=5).

continued

Fatty Acids	WC 1				
	Day 1	**Day 7**	**Day 8**	**Day 12**	**Day 17**
14:0	0.40 ± 0.12	0.49 ± 0.06	0.46 ± 0.08	0.51 ± 0.08	0.49 ± 0.09
16:0	11.31 ± 3.36	10.01 ± 0.37	10.50 ± 1.10	9.99 ± 2.19	10.04 ± 1.48
16:1n-7	0.54 ± 0.10	0.51 ± 0.08	0.52 ± 0.10	0.48 ± 0.08	0.65 ± 0.15
18:0	6.08 ± 0.81	7.23 ± 0.82	6.80 ± 1.39	6.30 ± 1.38	6.97 ± 1.40
18:1n-9	35.34 ± 5.77	35.94 ± 3.60	39.96 ± 7.09	39.57 ± 5.03	41.28 ± 7.23
18:1n-7	3.62 ± 0.33	3.61 ± 0.30	3.83 ± 0.48	3.41 ± 0.44	3.51 ± 0.24
18:2n-6	18.34 ± 3.24	18.98 ± 2.15	19.20 ± 2.70	18.52 ± 3.58	17.21 ± 2.22
20:3n-6	0.26 ± 0.12	0.30 ± 0.05	0.21 ± 0.04	0.33 ± 0.18	0.19 ± 0.02
20:4n-6	4.47 ± 1.02	5.02 ± 1.03	4.32 ± 1.66	4.88 ± 0.61	4.93 ± 2.12
22:4n-6	0.93 ± 0.09	1.00 ± 0.10	1.03 ± 0.26	1.04 ± 0.22	0.71 ± 0.09
22:5n-6	0.21 ± 0.07	0.24 ± 0.06	0.23 ± 0.06	0.31 ± 0.17	0.17 ± 0.04
18:3n-3	2.08 ± 0.50	2.02 ± 0.30	2.05 ± 0.34	1.89 ± 0.22	2.56 ± 0.34
20:5n-3	0.70 ± 0.19	0.54 ± 0.06	0.59 ± 0.14	0.58 ± 0.13	0.42 ± 0.05
22:5n-3	0.72 ± 0.22	0.86 ± 0.26	0.73 ± 0.35	0.76 ± 0.24	0.80 ± 0.36
22:6n-3	4.41 ± 1.15	3.35 ± 0.94	4.64 ± 0.84	4.79 ± 0.88	4.15 ± 1.10
Total SFAs	18.13 ± 3.24	18.23 ± 1.01	18.13 ± 1.25	17.17 ± 1.83	17.82 ± 2.82
Total MUFAs	39.81 ± 6.04	40.38 ± 3.93	44.54 ± 7.03	43.72 ± 4.85	45.71 ± 7.15
Total n-6	24.20 ± 3.49	25.53 ± 3.05	24.99 ± 4.38	25.08 ± 4.10	23.20 ± 3.18
Total n-3	7.92 ± 1.61	6.77 ± 1.34	8.00 ± 1.26	8.02 ± 0.98	7.93 ± 1.17
Total FAs (mg/g)	24.81 ± 4.14	23.40 ± 3.06	26.77 ± 5.19	25.70 ± 4.70	28.49 ± 4.33

Data are expressed as mean ± SD (n=5).

continued

Fatty Acids	WC 2				
	Day 18	**Day 24**	**Day 25**	**Day 29**	**Day 34**
14:0	0.41 ± 0.10	0.44 ± 0.06	0.42 ± 0.05	0.46 ± 0.04	0.34 ± 0.08
16:0	11.11 ± 1.84	9.26 ± 2.39	10.02 ± 1.39	10.73 ± 1.87	10.82 ± 0.93
16:1n-7	0.44 ± 0.05	0.58 ± 0.15	0.63 ± 0.29	0.65 ± 0.19	0.37 ± 0.10
18:0	6.95 ± 1.75	7.48 ± 1.12	7.44 ± 1.39	6.26 ± 1.42	7.43 ± 1.75
18:1n-9	40.58 ± 4.25	41.64 ± 6.88	43.36 ± 6.69	40.45 ± 6.25	41.12 ± 6.32
18:1n-7	3.76 ± 0.51	3.91 ± 0.70	4.03 ± 0.74	3.84 ± 0.30	4.24 ± 0.38
18:2n-6	18.72 ± 2.82	16.48 ± 2.14	17.55 ± 1.22	16.85 ± 3.50	17.50 ± 2.80
20:3n-6	0.27 ± 0.09	0.37 ± 0.06	0.30 ± 0.08	0.33 ± 0.25	0.39 ± 0.10
20:4n-6	4.39 ± 1.17	4.86 ± 1.90	4.78 ± 1.81	4.09 ± 0.95	4.90 ± 1.02
22:4n-6	0.77 ± 0.11	0.94 ± 0.10	0.85 ± 0.18	0.84 ± 0.06	0.75 ± 0.21
22:5n-6	0.15 ± 0.02	0.18 ± 0.05	0.18 ± 0.04	0.22 ± 0.06	0.20 ± 0.05
18:3n-3	2.26 ± 0.36	2.44 ± 0.22	2.52 ± 0.23	2.84 ± 0.43	2.07 ± 0.24
20:5n-3	0.53 ± 0.08	0.97 ± 0.21	0.55 ± 0.08	0.78 ± 0.13	0.72 ± 0.07
22:5n-3	0.70 ± 0.18	0.89 ± 0.29	0.63 ± 0.22	0.81 ± 0.13	0.74 ± 0.15
22:6n-3	4.82 ± 0.71	3.85 ± 0.70	3.93 ± 0.96	4.15 ± 0.93	4.13 ± 1.10
Total SFAs	18.96 ± 3.39	17.82 ± 2.30	18.17 ± 2.47	17.79 ± 1.51	19.10 ± 1.00
Total MUFAs	45.15 ± 4.72	46.39 ± 7.59	48.25 ± 6.90	45.15 ± 6.32	46.10 ± 6.58
Total n-6	24.31 ± 3.29	22.83 ± 2.14	23.67 ± 1.13	22.34 ± 4.15	23.74 ± 3.49
Total n-3	8.30 ± 0.87	8.14 ± 1.17	7.64 ± 0.67	8.58 ± 1.11	7.65 ± 1.30
Total FAs (mg/g)	25.20 ± 3.24	24.94 ± 5.32	25.48 ± 5.50	26.44 ± 4.99	25.87 ± 3.41

Data are expressed as mean ± SD (n=5).

Table 3.4 Fatty Acid Composition of carcass PL in rats of MF group

Fatty Acids	CTL		
	Day 0	Day 17	Day 34
14:0	0.39 ± 0.10	0.39 ± 0.14	0.38 ± 0.05
16:0	9.96 ± 1.74	9.13 ± 0.98	9.62 ± 1.11
16:1n-7	0.38 ± 0.07	0.42 ± 0.09	0.43 ± 0.11
18:0	6.29 ± 1.55	5.91 ± 1.43	5.81 ± 0.85
18:1n-9	32.59 ± 1.34	35.14 ± 3.46	33.69 ± 4.57
18:1n-7	3.03 ± 0.39	3.45 ± 0.52	3.46 ± 0.41
18:2n-6	17.87 ± 1.11	17.57 ± 1.25	16.67 ± 3.57
20:3n-6	0.29 ± 0.07	0.25 ± 0.10	0.32 ± 0.05
20:4n-6	4.08 ± 0.89	3.99 ± 1.24	4.18 ± 0.40
22:4n-6	0.83 ± 0.21	0.74 ± 0.25	0.82 ± 0.11
22:5n-6	0.17 ± 0.05	0.16 ± 0.04	0.15 ± 0.04
18:3n-3	2.04 ± 0.34	2.25 ± 0.47	2.16 ± 0.34
20:5n-3	0.69 ± 0.07	0.62 ± 0.20	0.71 ± 0.09
22:5n-3	0.71 ± 0.22	0.67 ± 0.39	0.73 ± 0.13
22:6n-3	3.81 ± 0.68	4.19 ± 1.47	4.43 ± 0.95
Total SFAs	16.94 ± 2.66	15.73 ± 2.13	16.14 ± 1.60
Total MUFAs	36.08 ± 1.57	39.15 ± 3.75	37.80 ± 4.74
Total n-6	23.23 ± 1.97	22.71 ± 2.58	22.14 ± 3.69
Total n-3	7.25 ± 1.08	7.73 ± 2.45	8.03 ± 1.18
Total FAs (mg/g)	17.04 ± 1.58	18.82 ± 3.06	18.50 ± 2.94

Data are expressed as mean ± SD (n=5).

continued

Fatty Acids	WC 1				
	Day 1	**Day 7**	**Day 8**	**Day 12**	**Day 17**
14:0	0.35 ± 0.10	0.42 ± 0.08	0.41 ± 0.10	0.46 ± 0.09	0.45 ± 0.10
16:0	10.04 ± 2.49	8.72 ± 1.20	9.06 ± 0.58	9.05 ± 2.62	9.19 ± 2.18
16:1n-7	0.54 ± 0.09	0.45 ± 0.12	0.45 ± 0.08	0.42 ± 0.05	0.59 ± 0.12
18:0	5.44 ± 0.76	6.24 ± 0.59	5.96 ± 1.57	5.59 ± 0.95	6.39 ± 1.81
18:1n-9	33.89 ± 5.83	31.44 ± 6.39	34.35 ± 4.15	35.50 ± 5.27	37.02 ± 3.97
18:1n-7	3.26 ± 0.57	3.15 ± 0.58	3.32 ± 0.52	3.04 ± 0.17	3.19 ± 0.46
18:2n-6	16.35 ± 2.56	16.36 ± 1.18	16.79 ± 3.55	16.74 ± 4.12	15.66 ± 2.88
20:3n-6	0.22 ± 0.09	0.26 ± 0.05	0.18 ± 0.05	0.30 ± 0.12	0.17 ± 0.03
20:4n-6	3.98 ± 0.77	4.29 ± 0.58	3.76 ± 1.39	4.36 ± 0.47	4.58 ± 1.36
22:4n-6	0.84 ± 0.15	0.87 ± 0.13	0.89 ± 0.20	0.93 ± 0.23	0.87 ± 0.29
22:5n-6	0.19 ± 0.07	0.20 ± 0.05	0.20 ± 0.07	0.29 ± 0.12	0.16 ± 0.05
18:3n-3	1.88 ± 0.50	1.75 ± 0.28	1.78 ± 0.37	1.70 ± 0.33	2.33 ± 0.43
20:5n-3	0.62 ± 0.13	0.55 ± 0.10	0.51 ± 0.10	0.52 ± 0.09	0.47 ± 0.13
22:5n-3	0.65 ± 0.25	0.73 ± 0.18	0.64 ± 0.20	0.68 ± 0.21	0.75 ± 0.40
22:6n-3	4.01 ± 1.23	2.85 ± 0.61	4.04 ± 0.91	4.33 ± 1.05	3.78 ± 1.15
Total SFAs	16.14 ± 2.21	15.82 ± 1.72	15.73 ± 1.77	15.44 ± 2.49	16.32 ± 4.03
Total MUFAs	37.99 ± 6.45	35.32 ± 7.11	38.32 ± 3.98	39.18 ± 5.08	41.04 ± 3.72
Total n-6	21.58 ± 2.62	21.98 ± 1.46	21.82 ± 4.86	22.62 ± 4.68	21.44 ± 3.64
Total n-3	7.15 ± 1.76	5.88 ± 0.90	6.97 ± 1.32	7.23 ± 1.37	7.34 ± 1.67
Total FAs (mg/g)	17.49 ± 2.76	16.21 ± 3.33	17.61 ± 3.44	17.93 ± 3.26	18.35 ± 3.07

Data are expressed as mean ± SD (n=5).

continued

Fatty Acids	WC 2				
	Day 18	**Day 24**	**Day 25**	**Day 29**	**Day 34**
14:0	0.36 ± 0.07	0.39 ± 0.09	0.37 ± 0.02	0.42 ± 0.07	0.31 ± 0.08
16:0	9.75 ± 1.01	8.20 ± 2.13	8.90 ± 1.48	9.83 ± 2.28	9.76 ± 1.30
16:1n-7	0.39 ± 0.07	0.52 ± 0.11	0.56 ± 0.26	0.43 ± 0.07	0.33 ± 0.08
18:0	6.10 ± 1.31	6.71 ± 1.45	6.65 ± 1.53	6.46 ± 1.22	6.73 ± 1.79
18:1n-9	36.24 ± 7.13	37.06 ± 7.09	38.22 ± 3.93	36.99 ± 7.66	37.03 ± 6.53
18:1n-7	3.37 ± 0.77	3.48 ± 0.69	3.59 ± 0.78	3.50 ± 0.48	3.82 ± 0.35
18:2n-6	16.55 ± 2.65	14.56 ± 1.26	15.61 ± 1.98	15.23 ± 2.94	15.81 ± 2.95
20:3n-6	0.24 ± 0.08	0.33 ± 0.05	0.35 ± 0.09	0.32 ± 0.27	0.35 ± 0.10
20:4n-6	3.82 ± 0.72	4.36 ± 1.80	4.31 ± 1.77	3.79 ± 0.44	4.42 ± 1.01
22:4n-6	0.69 ± 0.15	0.84 ± 0.10	0.76 ± 0.17	0.76 ± 0.05	0.70 ± 0.15
22:5n-6	0.13 ± 0.02	0.16 ± 0.06	0.25 ± 0.07	0.20 ± 0.04	0.18 ± 0.04
18:3n-3	2.01 ± 0.39	2.17 ± 0.30	2.25 ± 0.33	2.59 ± 0.49	1.88 ± 0.36
20:5n-3	0.47 ± 0.10	0.87 ± 0.22	0.49 ± 0.09	0.71 ± 0.14	0.65 ± 0.10
22:5n-3	0.62 ± 0.18	0.78 ± 0.22	0.58 ± 0.24	0.74 ± 0.17	0.67 ± 0.15
22:6n-3	4.26 ± 0.64	3.43 ± 0.69	3.49 ± 0.89	3.75 ± 0.72	3.75 ± 1.12
Total SFAs	16.64 ± 2.02	15.87 ± 2.45	16.18 ± 2.83	17.03 ± 2.55	17.25 ± 2.04
Total MUFAs	40.31 ± 7.95	41.28 ± 7.78	42.57 ± 4.22	41.12 ± 8.17	41.51 ± 6.79
Total n-6	21.44 ± 2.63	20.26 ± 1.78	21.28 ± 2.87	20.29 ± 3.14	21.46 ± 3.77
Total n-3	7.35 ± 0.96	7.25 ± 1.20	6.80 ± 0.98	7.78 ± 1.06	6.94 ± 1.51
Total FAs (mg/g)	17.49 ± 3.35	16.66 ± 3.84	18.54 ± 2.33	18.67 ± 3.75	18.84 ± 2.34

Data are expressed as mean ± SD (n=5).

3.4.7.1.3 Carcass Triglycerides (TG)

Unlike the carcass phospholipids, the fatty acid composition of carcass triglycerides changed in WC rats during the 2 WCs. The concentration of 18:2n-6 and 18:3n-3 gradually decreased in the WC rats fed both HF and MF diet compared with CTL rats fed the same diet. During the two refeeding periods of each cycle, the content of 16:0 and 18:0 of carcass TG was significantly higher than that in the CTL rats at the end of two cycles. Furthermore, the content of 16:1n-7 in WC rats fed MF diet was also increased after two WCs (Figures 3.26, 3.27, 3.29 and 3.30).

3.4.7.1.4 Carcass Free Fatty Acids (FFA)

Some of the FFA in carcass fluctuated in WC rats during the experiment. However, after two WCs, the pattern of the fatty acid composition in WC rats was similar to that in CTL rats regardless of the type of diet used (Tables 3.5 and 3.6).

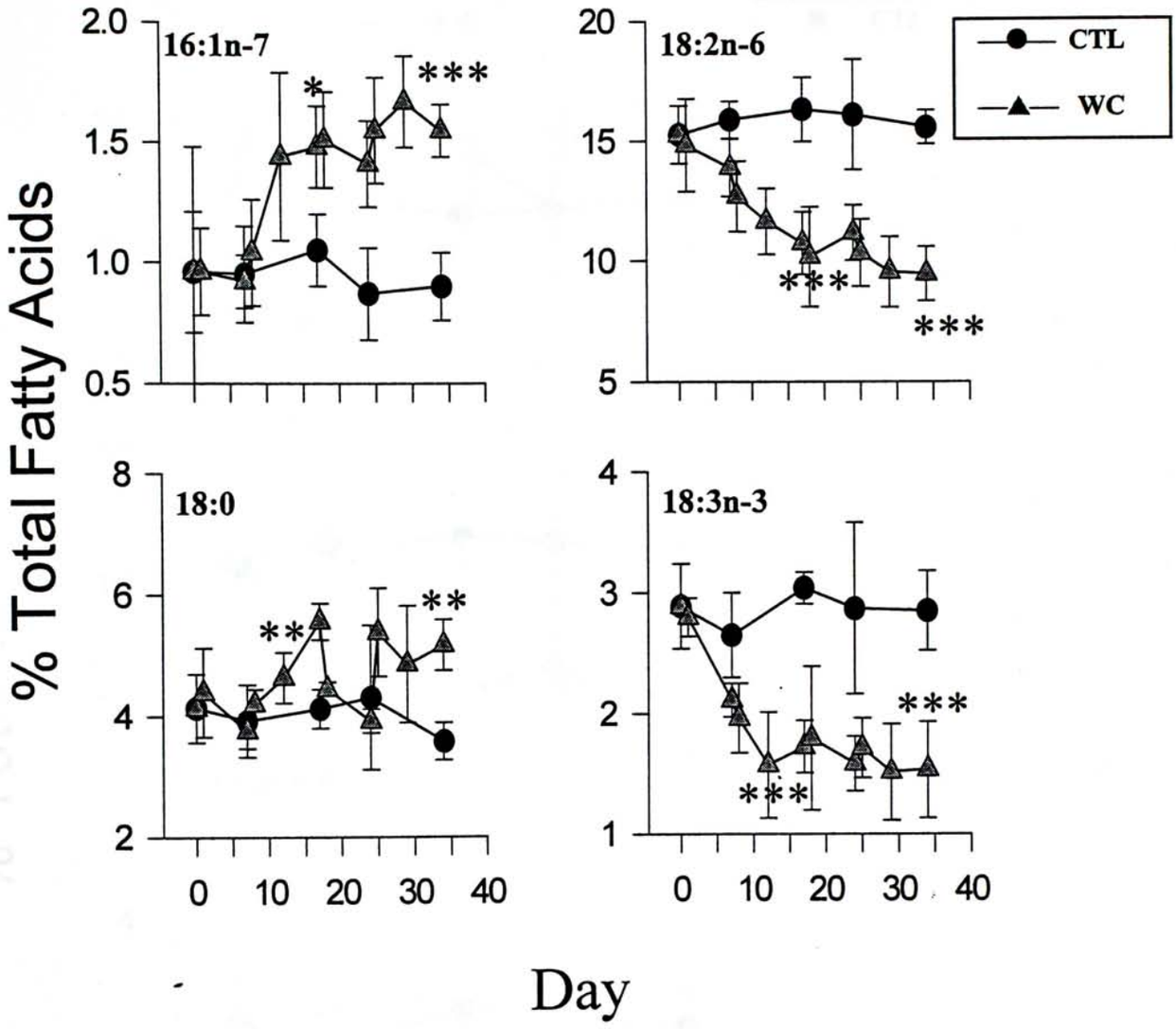


Figure 3.27 Time-course changes in individual fatty acid in carcass TG in rats fed HF diet. (*p<0.05, **p<0.01 and ***p<0.001, difference between WC and CTL rats.)

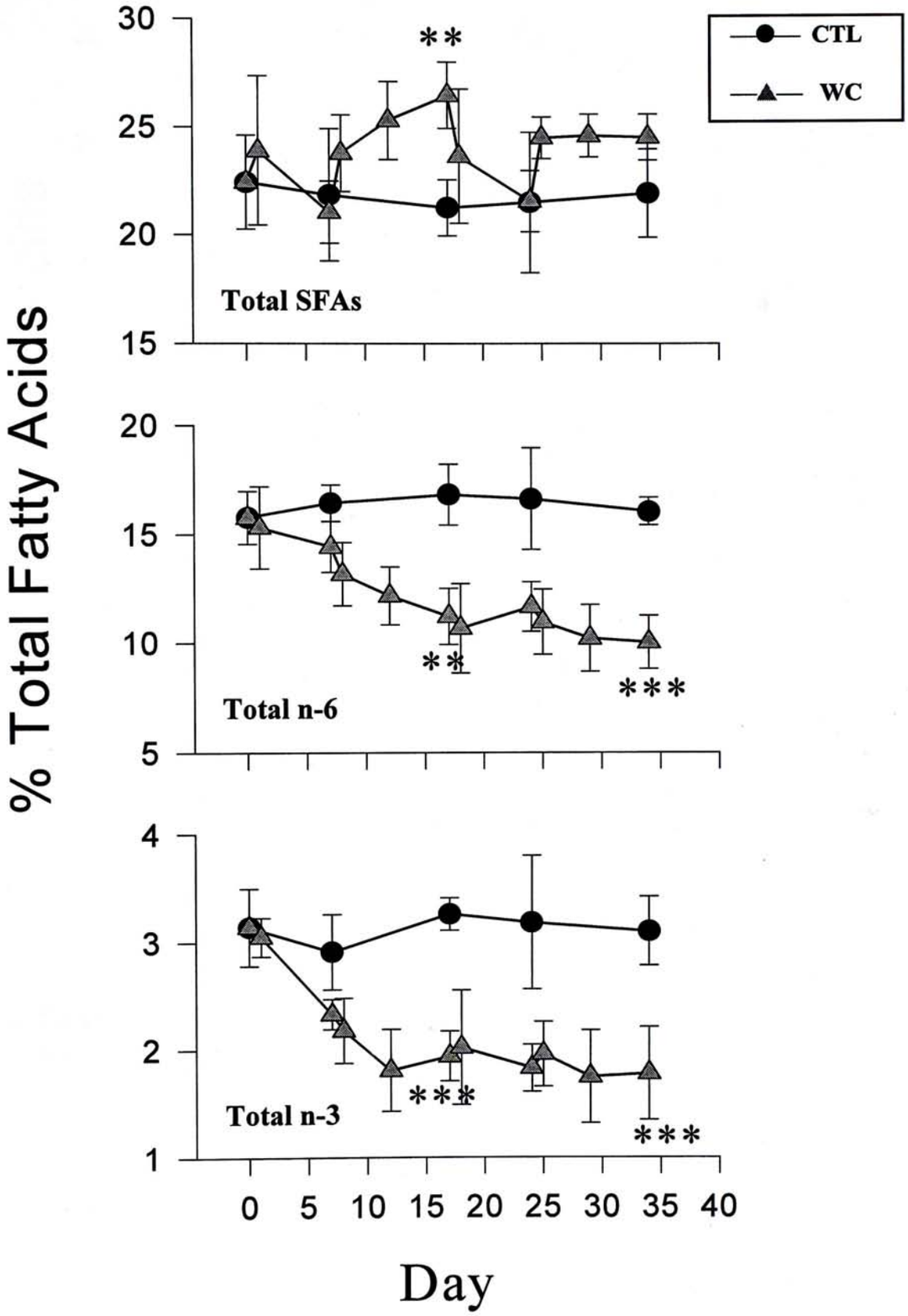


Figure 3.28 Time-course changes in total SFAs, total n-6 and total n-3 in carcass TG in rats fed HF diet. (**p<0.01 and ***p<0.001, difference between WC and CTL rats.)

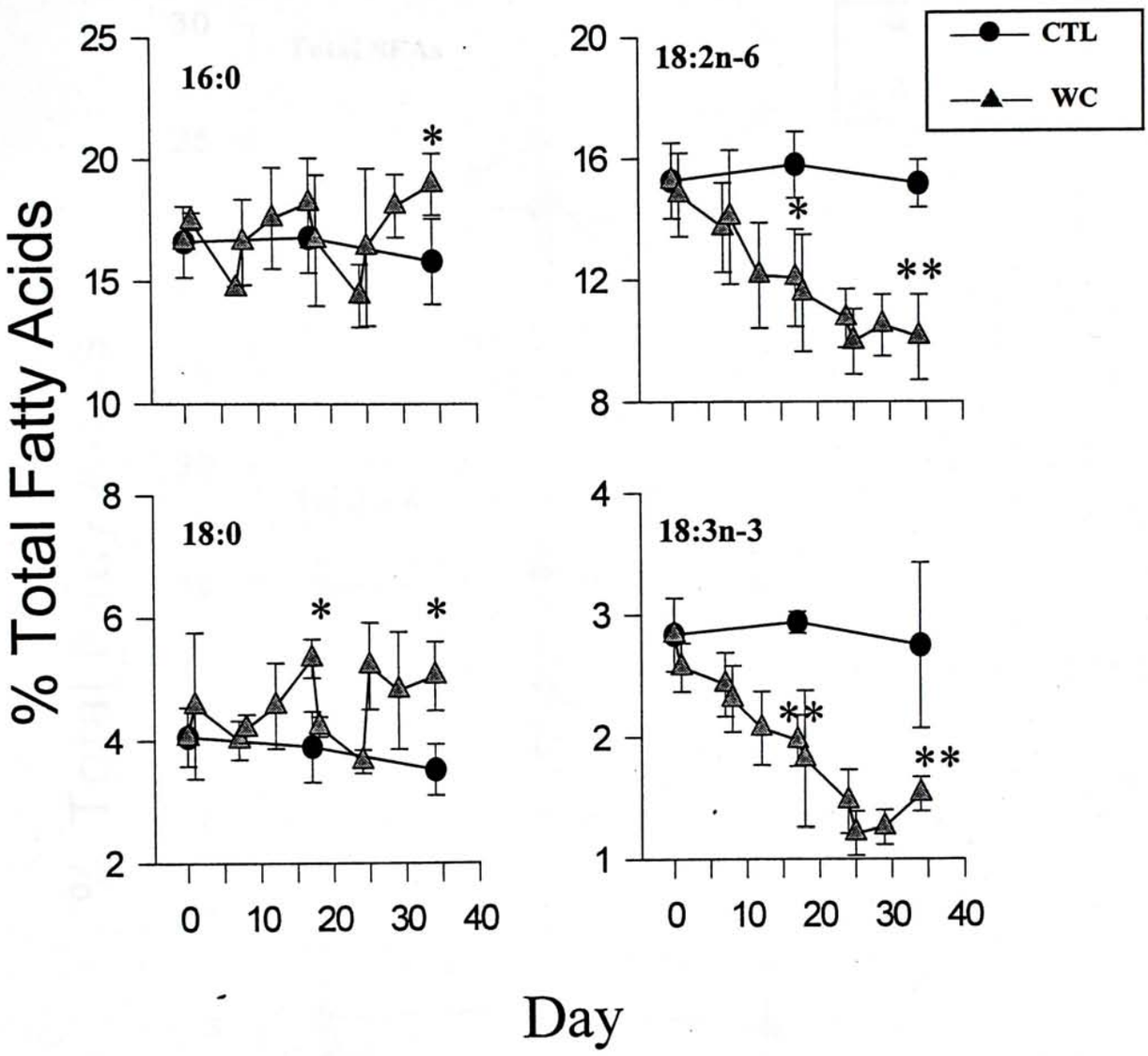


Figure 3.29 Time-course changes in individual fatty acid in carcass TG in rats fed MF diet. (*p<0.05 and **p<0.01, difference between WC and CTL rats.)

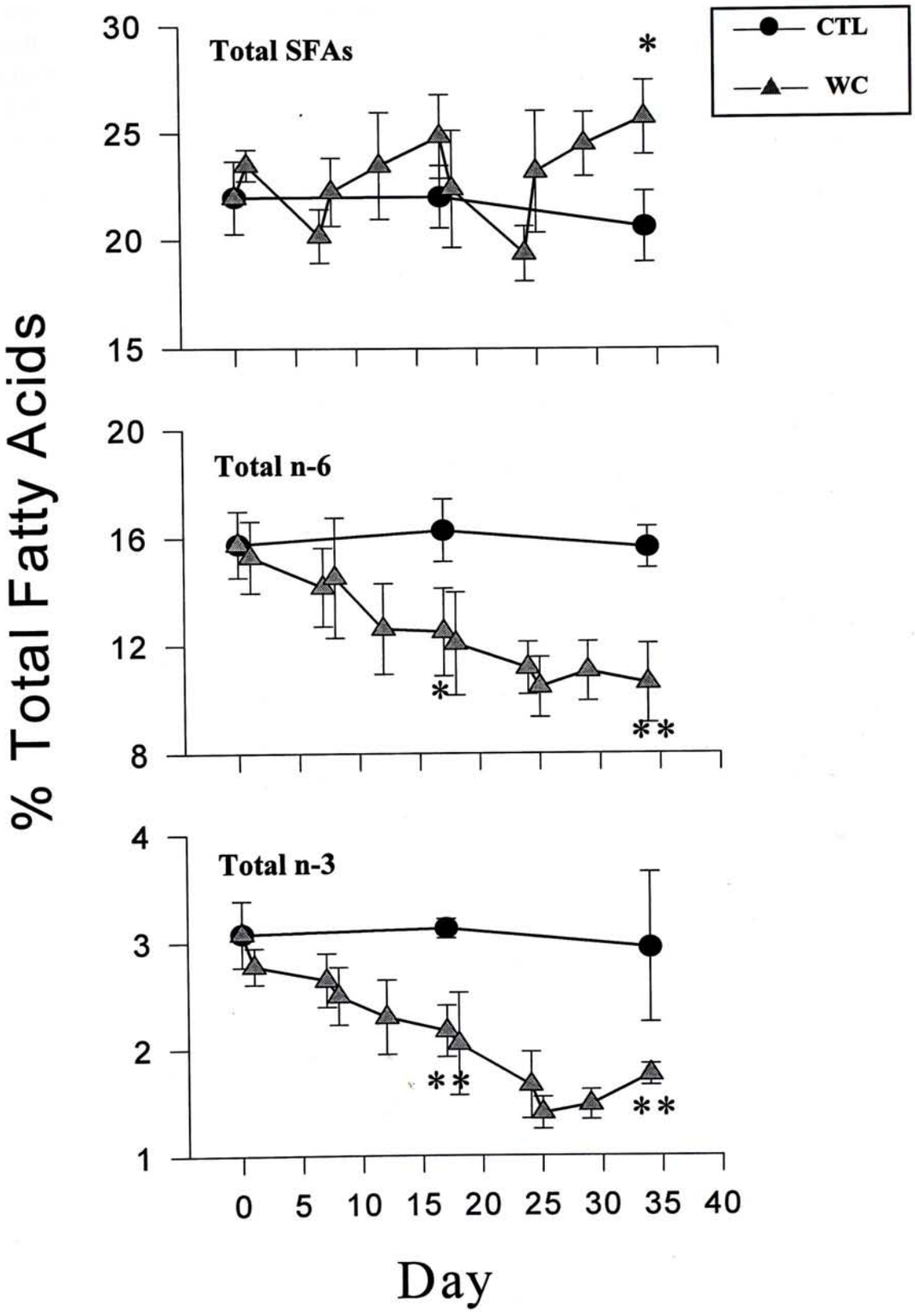


Figure 3.30 Time-course changes in total SFAs, total n-6 and total n-3 in carcass TG in rats fed MF diet. (*$p<0.05$ and **$p<0.01$, difference between WC and CTL rats.)

Table 3.5 Fatty Acid Composition of carcass FFA in rats of HF group

Fatty Acids	CTL				
	Day 0	**Day 7**	**Day 17**	**Day 24**	**Day 34**
14:0	0.98 ± 0.16	0.94 ± 0.06	0.74 ± 0.09	0.83 ± 0.04	0.64 ± 0.04
16:0	14.70 ± 1.75	12.91 ± 1.12	11.59 ± 0.78	12.18 ± 1.03	10.90 ± 0.39
16:1n-7	1.60 ± 0.29	1.81 ± 0.34	1.25 ± 0.14	1.29 ± 0.30	1.33 ± 0.12
18:0	5.05 ± 0.85	4.05 ± 0.90	3.98 ± 0.41	3.44 ± 1.07	4.51 ± 0.38
18:1n-9	51.13 ± 1.14	48.96 ± 2.73	54.86 ± 1.78	54.32 ± 2.92	57.07 ± 1.35
18:1n-7	4.24 ± 0.10	3.75 ± 0.42	4.27 ± 0.30	4.79 ± 0.66	4.10 ± 0.34
18:2n-6	13.07 ± 0.54	13.29 ± 0.80	12.33 ± 1.01	11.80 ± 4.22	11.98 ± 0.75
20:3n-6	0.16 ± 0.02	0.14 ± 0.02	0.05 ± 0.02	0.60 ± 0.11	0.12 ± 0.01
20:4n-6	0.67 ± 0.32	0.51 ± 0.12	0.57 ± 0.13	0.77 ± 0.13	0.61 ± 0.14
22:4n-6	0.07 ± 0.01	0.13 ± 0.03	0.14 ± 0.04	0.13 ± 0.04	0.07 ± 0.01
22:5n-6	0.20 ± 0.03	0.21 ± 0.06	0.23 ± 0.06	0.29 ± 0.09	0.06 ± 0.01
18:3n-3	2.41 ± 0.32	2.77 ± 0.26	2.23 ± 0.25	2.67 ± 0.55	2.27 ± 0.10
20:5n-3	0.15 ± 0.02	0.11 ± 0.05	0.12 ± 0.02	0.16 ± 0.02	0.13 ± 0.03
22:5n-3	0.13 ± 0.02	0.12 ± 0.02	0.14 ± 0.04	0.18 ± 0.01	0.09 ± 0.02
22:6n-3	0.38 ± 0.04	0.38 ± 0.10	0.28 ± 0.06	0.33 ± 0.05	0.17 ± 0.04
Total SFAs	21.24 ± 1.77	18.17 ± 2.03	16.89 ± 1.19	16.95 ± 0.98	16.53 ± 0.61
Total MUFAs	57.88 ± 1.16	55.52 ± 2.83	61.52 ± 2.06	61.15 ± 3.11	63.67 ± 1.43
Total n-6	14.18 ± 0.81	14.28 ± 0.86	13.32 ± 1.05	13.58 ± 4.24	12.84 ± 0.66
Total n-3	3.06 ± 0.36	3.38 ± 0.29	2.77 ± 0.15	3.34 ± 0.53	2.68 ± 0.10
Total FAs (mg/g)	14.86 ± 3.34	14.43 ± 3.26	15.08 ± 0.88	16.11 ± 1.17	15.70 ± 1.46

Data are expressed as mean ± SD (n=5).

continued

Fatty Acids	WC 1				
	Day 1	**Day 7**	**Day 8**	**Day 12**	**Day 17**
14:0	0.98 ± 0.28	0.93 ± 0.15	0.73 ± 0.10	0.78 ± 0.18	0.79 ± 0.17
16:0	13.34 ± 2.06	11.36 ± 0.83	10.52 ± 1.11	10.77 ± 1.01	10.05 ± 1.40
16:1n-7	1.83 ± 0.16	1.23 ± 0.21	1.07 ± 0.26	1.07 ± 0.11	1.04 ± 0.23
18:0	4.48 ± 0.33	4.90 ± 0.33	4.26 ± 0.50	4.29 ± 0.15	3.88 ± 0.19
18:1n-9	51.18 ± 2.31	52.03 ± 1.28	54.08 ± 2.36	53.48 ± 2.25	52.16 ± 2.35
18:1n-7	4.20 ± 0.19	4.28 ± 0.33	4.35 ± 0.21	4.20 ± 0.30	4.23 ± 0.19
18:2n-6	11.11 ± 1.66	11.31 ± 0.93	11.95 ± ± 2.01	13.30 ± 1.98	13.97 ± 1.21
20:3n-6	0.09 ± 0.02	0.12 ± 0.03	0.12 ± 0.03	0.14 ± 0.08	0.11 ± 0.06
20:4n-6	0.71 ± 0.27	0.89 ± 0.21	1.07 ± 0.73	0.57 ± 0.33	0.80 ± 0.37
22:4n-6	0.08 ± 0.02	0.13 ± 0.02	0.11 ± 0.04	0.12 ± 0.01	0.10 ± 0.03
22:5n-6	0.13 ± 0.04	0.19 ± 0.01	0.15 ± 0.03	0.25 ± 0.08	0.29 ± 0.06
18:3n-3	2.27 ± 0.50	2.19 ± 0.39	2.38 ± 0.53	2.94 ± 0.73	2.10 ± 0.50
20:5n-3	0.13 ± 0.03	0.17 ± 0.03	0.15 ± 0.02	0.14 ± 0.02	0.14 ± 0.04
22:5n-3	0.09 ± 0.02	0.17 ± 0.04	0.14 ± 0.06	0.17 ± 0.02	0.18 ± 0.04
22:6n-3	0.27 ± 0.08	0.40 ± 0.09	0.41 ± 0.33	0.25 ± 0.07	0.33 ± 0.08
Total SFAs	19.31 ± 2.40	17.58 ± 1.00	15.91 ± 1.14	16.29 ± 0.94	15.10 ± 1.40
Total MUFAs	58.11 ± 2.44	58.48 ± 1.26	60.27 ± 2.43	59.66 ± 2.17	58.11 ± 2.60
Total n-6	12.13 ± 1.85	12.63 ± 0.91	13.41 ± 2.43	14.39 ± 2.19	15.27 ± 1.32
Total n-3	2.75 ± 0.57	2.93 ± 0.49	3.08 ± 0.75	3.50 ± 0.77	2.74 ± 0.46
Total FAs (mg/g)	15.12 ± 2.91	18.67 ± 2.23	16.49 ± 5.18	17.04 ± 3.06	15.41 ± 2.55

Data are expressed as mean ± SD (n=5).

continued

Fatty Acids	WC 2				
	Day 18	**Day 24**	**Day 25**	**Day 29**	**Day 34**
14:0	0.81 ± 0.18	0.80 ± 0.42	0.80 ± 0.14	0.68 ± 0.06	0.71 ± 0.10
16:0	11.73 ± 0.52	11.11 ± 1.10	11.82 ± 1.16	11.44 ± 1.32	11.10 ± 1.60
16:1n-7	1.41 ± 0.08	1.63 ± 0.28	1.24 ± 0.11	1.44 ± 0.39	1.38 ± 0.42
18:0	3.76 ± 0.24	3.73 ± 0.90	3.60 ± 0.97	4.13 ± 0.73	4.82 ± 0.57
18:1n-9	52.69 ± 1.66	49.97 ± 4.65	56.20 ± 3.78	49.93 ± 1.81	51.86 ± 3.65
18:1n-7	4.58 ± 0.15	4.60 ± 0.99	5.07 ± 0.48	4.95 ± 1.00	4.38 ± 0.63
18:2n-6	13.36 ± 1.56	11.66 ± 2.24	11.44 ± 1.41	11.46 ± 1.44	11.85 ± 1.07
20:3n-6	0.24 ± 0.15	0.32 ± 0.08	0.53 ± 0.07	0.52 ± 0.12	0.17 ± 0.06
20:4n-6	0.76 ± 0.30	0.83 ± 0.30	0.83 ± 0.28	0.89 ± 0.10	0.94 ± 0.56
22:4n-6	0.10 ± 0.02	0.13 ± 0.03	0.11 ± 0.03	0.11 ± 0.02	0.07 ± 0.03
22:5n-6	0.26 ± 0.03	0.27 ± 0.03	0.21 ± 0.04	0.23 ± 0.07	0.08 ± 0.01
18:3n-3	2.23 ± 0.36	2.31 ± 0.65	2.37 ± 0.50	2.24 ± 0.41	2.23 ± 0.29
20:5n-3	0.18 ± 0.05	0.20 ± 0.06	0.16 ± 0.04	0.13 ± 0.02	0.19 ± 0.08
22:5n-3	0.12 ± 0.03	0.15 ± 0.03	0.17 ± 0.04	0.15 ± 0.03	0.09 ± 0.03
22:6n-3	0.36 ± 0.05	0.47 ± 0.10	0.31 ± 0.04	0.23 ± 0.05	0.21 ± 0.08
Total SFAs	16.73 ± 0.85	16.26 ± 1.13	17.02 ± 1.09	16.90 ± 1.78	17.08 ± 1.63
Total MUFAs	59.29 ± 1.81	56.45 ± 5.55	62.76 ± 4.11	57.13 ± 2.28	57.82 ± 4.08
Total n-6	14.72 ± 1.74	13.20 ± 2.53	13.12 ± 1.24	13.21 ± 1.33	13.10 ± 1.44
Total n-3	2.90 ± 0.36	3.12 ± 0.56	3.01 ± 0.53	2.75 ± 0.45	2.72 ± 0.31
Total FAs (mg/g)	15.23 ± 2.50	18.85 ± 2.82	17.31 ± 2.23	15.23 ± 5.90	15.97 ± 3.52

Data are expressed as mean ± SD (n=5).

Table 3.6 Fatty Acid Composition of carcass FFA in rats of MF group

Fatty Acids	**CTL**		
	Day 0	**Day 17**	**Day 34**
14:0	1.08 ± 0.18	1.03 ± 0.10	1.03 ± 0.12
16:0	16.04 ± 1.03	15.84 ± 0.82	14.98 ± 1.20
16:1n-7	1.77 ± 0.32	1.55 ± 0.20	1.33 ± 0.15
18:0	5.59 ± 0.95	5.41 ± 0.50	4.84 ± 0.41
18:1n-9	46.01 ± 1.22	50.95 ± 4.24	53.14 ± 5.02
18:1n-7	4.69 ± 0.32	4.72 ± 0.34	4.54 ± 0.37
18:2n-6	14.46 ± 0.60	13.65 ± 1.11	13.26 ± 0.82
20:3n-6	0.18 ± 0.03	0.16 ± 0.02	0.14 ± 0.04
20:4n-6	0.75 ± 0.16	0.73 ± 0.14	0.67 ± 0.16
22:4n-6	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.02
22:5n-6	0.21 ± 0.01	0.22 ± 0.01	0.21 ± 0.02
18:3n-3	2.66 ± 0.36	2.47 ± 0.27	2.52 ± 0.49
20:5n-3	0.16 ± 0.02	0.13 ± 0.03	0.17 ± 0.03
22:5n-3	0.14 ± 0.02	0.14 ± 0.01	0.14 ± 0.02
22:6n-3	0.42 ± 0.05	0.41 ± 0.06	0.39 ± 0.05
Total SFAs	23.27 ± 1.39	22.91 ± 1.30	21.37 ± 1.63
Total MUFAs	53.48 ± 1.47	58.48 ± 4.35	60.31 ± 4.95
Total n-6	15.68 ± 0.73	14.84 ± 1.21	14.35 ± 0.75
Total n-3	3.38 ± 0.40	3.15 ± 0.29	3.22 ± 0.50
Total FAs (mg/g)	12.96 ± 3.48	12.73 ± 2.07	13.31 ± 2.65

Data are expressed as mean ± SD (n=5).

continued

Fatty Acids	WC 1				
	Day 1	**Day 7**	**Day 8**	**Day 12**	**Day 17**
14:0	1.09 ± 0.31	1.03 ± 0.13	1.11 ± 0.11	1.06 ± 0.30	1.10 ± 0.14
16:0	14.86 ± 3.48	15.48 ± 1.10	16.06 ± 1.92	15.07 ± 1.10	15.10 ± 1.37
16:1n-7	2.04 ± 0.40	1.51 ± 0.22	1.50 ± 0.30	1.39 ± 0.13	1.36 ± 0.25
18:0	4.99 ± 0.35	5.47 ± 0.38	5.26 ± 0.58	5.43 ± 0.33	5.36 ± 0.20
18:1n-9	46.24 ± 2.55	47.19 ± 1.38	49.28 ± 2.79	48.60 ± 2.50	49.04 ± 3.19
18:1n-7	4.68 ± 0.22	4.78 ± 0.38	4.83 ± 0.26	4.65 ± 0.34	4.67 ± 0.20
18:2n-6	13.87 ± 2.02	12.28 ± 1.28	11.61 ± 2.46	11.80 ± 2.04	11.43 ± 0.81
20:3n-6	0.15 ± 0.02	0.13 ± 0.03	0.14 ± 0.03	0.16 ± 0.09	0.12 ± 0.06
20:4n-6	0.79 ± 0.29	0.89 ± 0.26	0.97 ± 0.38	0.73 ± 0.32	0.88 ± 0.41
22:4n-6	0.09 ± 0.02	0.09 ± 0.02	0.12 ± 0.04	0.14 ± 0.02	0.11 ± 0.02
22:5n-6	0.22 ± 0.01	0.21 ± 0.01	0.18 ± 0.03	0.20 ± 0.04	0.21 ± 0.02
18:3n-3	2.15 ± 0.54	2.04 ± 0.33	2.02 ± 0.33	1.97 ± 0.42	1.92 ± 0.18
20:5n-3	0.15 ± 0.04	0.19 ± 0.03	0.13 ± 0.01	0.16 ± 0.02	0.15 ± 0.04
22:5n-3	0.12 ± 0.02	0.19 ± 0.05	0.15 ± 0.07	0.19 ± 0.02	0.17 ± 0.04
22:6n-3	0.30 ± 0.09	0.52 ± 0.10	0.46 ± 0.37	0.38 ± 0.07	0.37 ± 0.14
Total SFAs	21.51 ± 3.63	22.42 ± 1.15	22.87 ± 1.94	22.07 ± 1.33	21.97 ± 1.67
Total MUFAs	53.95 ± 2.85	54.54 ± 1.34	56.47 ± 2.81	55.63 ± 2.41	55.82 ± 3.11
Total n-6	15.13 ± 2.29	13.61 ± 1.45	13.02 ± 2.72	13.03 ± 2.16	12.76 ± 1.20
Total n-3	2.71 ± 0.59	2.95 ± 0.30	2.76 ± 0.62	2.69 ± 0.45	2.61 ± 0.13
Total FAs (mg/g)	13.02 ± 2.43	15.80 ± 2.22	13.14 ± 2.72	12.59 ± 2.31	12.28 ± 3.57

Data are expressed as mean ± SD (n=5).

continued

Fatty Acids	WC 2				
	Day 18	**Day 24**	**Day 25**	**Day 29**	**Day 34**
14:0	0.98 ± 0.49	0.90 ± 0.25	0.78 ± 0.12	0.75 ± 0.07	0.85 ± 0.13
16:0	15.02 ± 0.57	13.93 ± 0.80	14.91 ± 1.68	14.43 ± 2.22	14.92 ± 2.18
16:1n-7	1.56 ± 0.09	1.62 ± 0.51	1.54 ± 0.08	1.57 ± 0.20	1.72 ± 0.54
18:0	5.34 ± 0.42	5.47 ± 0.80	5.21 ± 1.12	5.79 ± 0.88	5.34 ± 0.62
18:1n-9	47.60 ± 4.04	48.88 ± 1.18	51.56 ± 4.15	46.96 ± 4.60	47.18 ± 2.08
18:1n-7	4.82 ± 0.31	5.09 ± 1.09	5.59 ± 0.52	5.48 ± 1.10	5.57 ± 0.67
18:2n-6	10.54 ± 1.86	10.78 ± 2.46	10.51 ± 1.64	10.04 ± 1.39	10.28 ± 1.74
20:3n-6	0.18 ± 0.05	0.23 ± 0.07	0.29 ± 0.09	0.23 ± 0.05	0.21 ± 0.07
20:4n-6	0.84 ± 0.33	0.92 ± 0.25	0.99 ± 0.30	0.92 ± 0.19	1.04 ± 0.62
22:4n-6	0.11 ± 0.02	0.12 ± 0.03	0.22 ± 0.06	0.20 ± 0.02	0.14 ± 0.05
22:5n-6	0.18 ± 0.03	0.18 ± 0.04	0.21 ± 0.04	0.25 ± 0.08	0.29 ± 0.04
18:3n-3	1.82 ± 0.40	1.75 ± 0.25	1.81 ± 0.33	1.60 ± 0.44	1.71 ± 0.32
20:5n-3	0.20 ± 0.06	0.25 ± 0.07	0.38 ± 0.16	0.27 ± 0.06	0.33 ± 0.19
22:5n-3	0.14 ± 0.03	0.16 ± 0.05	0.19 ± 0.04	0.17 ± 0.02	0.17 ± 0.03
22:6n-3	0.40 ± 0.06	0.56 ± 0.17	0.53 ± 0.18	0.51 ± 0.05	0.59 ± 0.29
Total SFAs	21.81 ± 0.52	20.98 ± 1.35	21.76 ± 1.26	21.69 ± 1.43	21.62 ± 2.04
Total MUFAs	54.65 ± 4.33	55.86 ± 0.36	58.98 ± 4.56	54.91 ± 3.95	54.69 ± 2.45
Total n-6	11.85 ± 2.16	12.23 ± 2.46	12.22 ± 1.62	11.63 ± 1.36	11.95 ± 2.31
Total n-3	2.55 ± 0.39	2.72 ± 0.35	2.91 ± 0.44	2.54 ± 0.49	2.80 ± 0.75
Total FAs (mg/g)	13.46 ± 2.41	16.71 ± 3.47	14.27 ± 2.39	13.35 ± 5.65	12.92 ± 3.25

Data are expressed as mean ± SD (n=5).

3.4.7.2 Adipose Fatty Acids

During food restrictions, 18:2n-6, 18:3n-3, eicosapentaenoic acid (20:5n-3), docosapentaenoic acid (22:5n-3) and docosahexaenoic acid (22:6n-3), in the adipose tissue of WC rats in HF groups were significantly decreased during food restriction periods (Table 3.7, Figures 3.31 and 3.32). In the second food restriction period, 14:0, 16:0 and 18:0 were significantly increased in WC rats fed a HF diet. At the end of the first and second cycles, the content of 18:2n-6 and 20:5n-3 were considerably decreased. Furthermore, 16:0 was significantly increased in the WC rats at the end of the second cycle.

In MF group, 18:2n-6, 18:3n-3, 20:3n-6, 20:4n-6, 224n-6, 20:5n-3 and 22:5n-3 were significantly lower in WC rats than in CTL rats during food restrictions (Table 3.8, Figures 3.33 and 3.34). At the end of the weight cycles, 16:0 was significantly increased in WC rats, whereas 18:2n-6 was markedly reduced. Besides, at the end of the second cycle, 18:3n-3 and 20:4n-6 were also reduced in the WC rats.

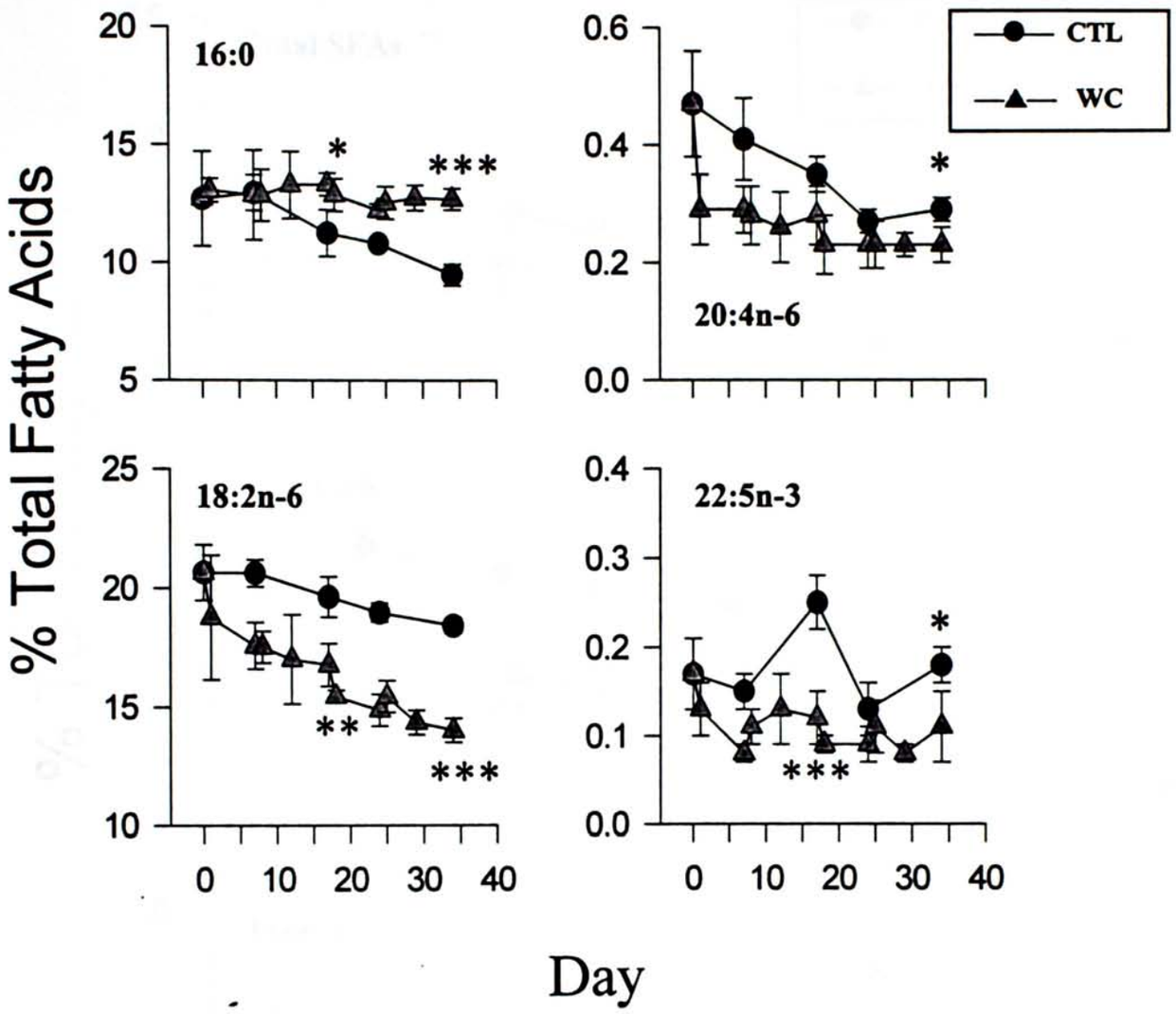


Figure 3.31 Time-course changes in individual fatty acid in adipose tissue in rats fed HF diet. (*p<0.05, **p<0.01 and ***p<0.001, difference between WC and CTL rats.)

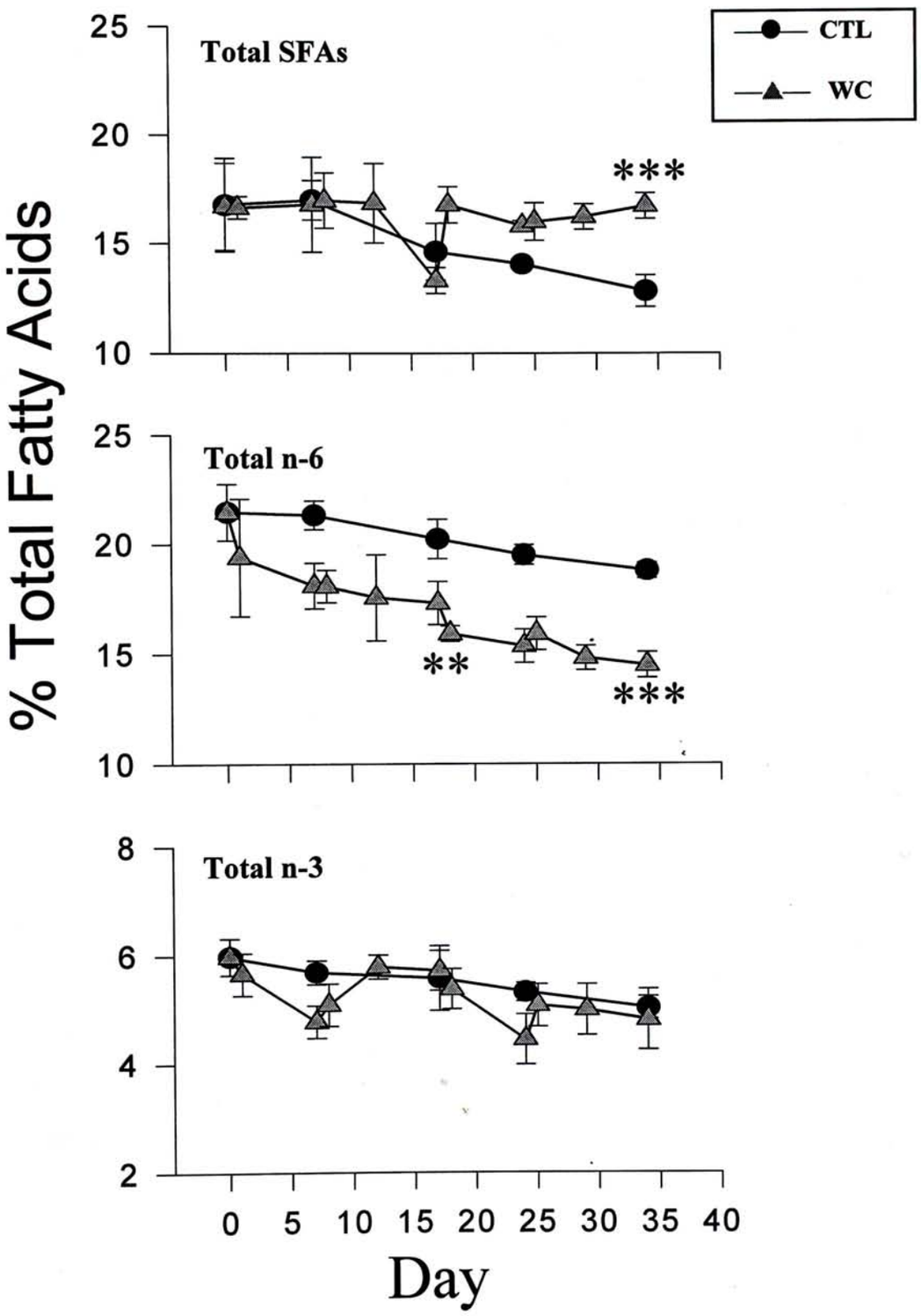


Figure 3.32 Time-course changes in total SFAs, total n-6 and total n-3 in adipose tissue in rats fed HF diet. (**p<0.01 and ***p<0.001, difference between WC and CTL rats.)

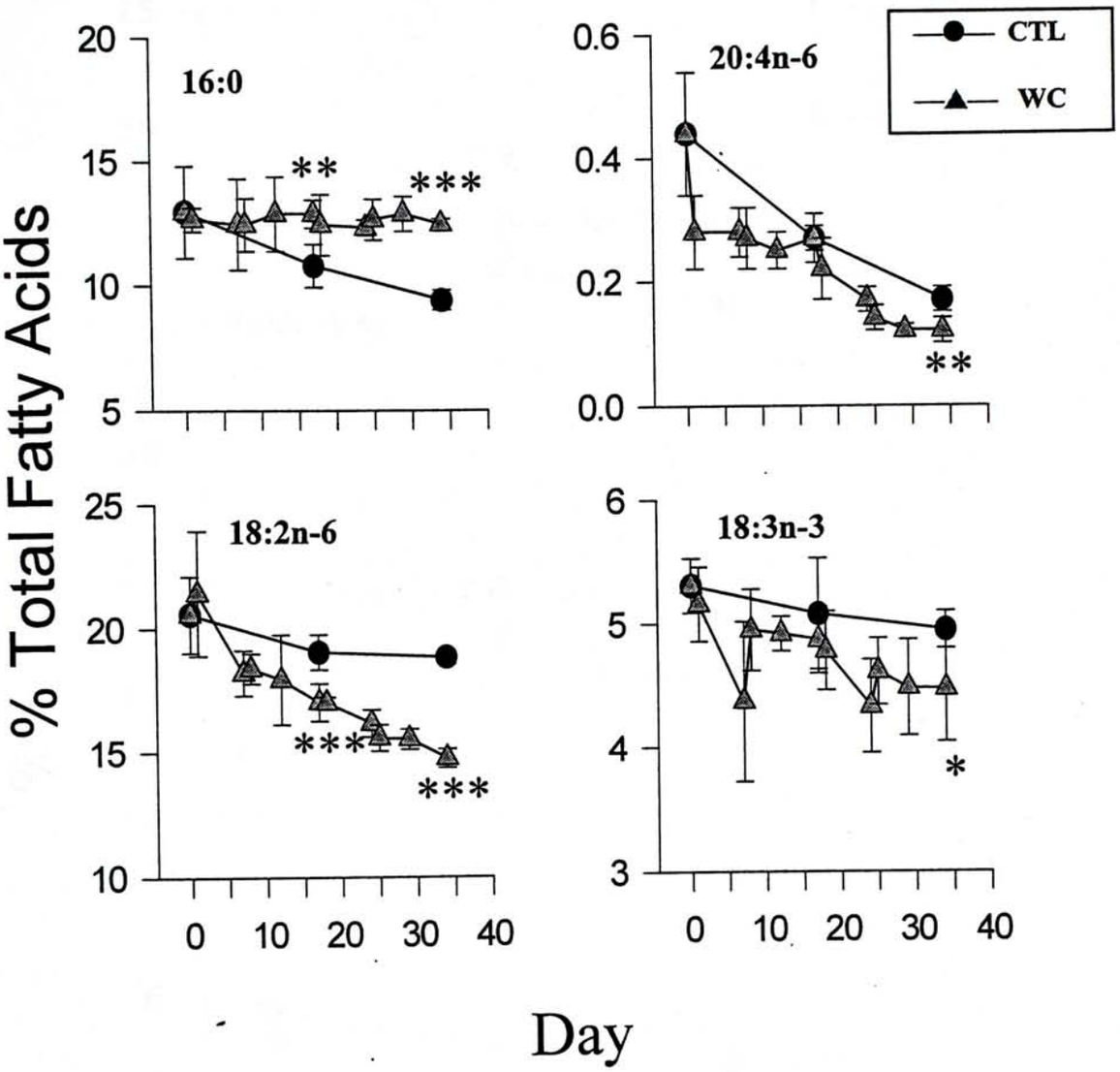


Figure 3.33 Time-course changes in individual fatty acid in adipose tissue in rats fed MF diet. (*p<0.05, **p<0.01 and ***p<0.001, difference between WC and CTL rats.)

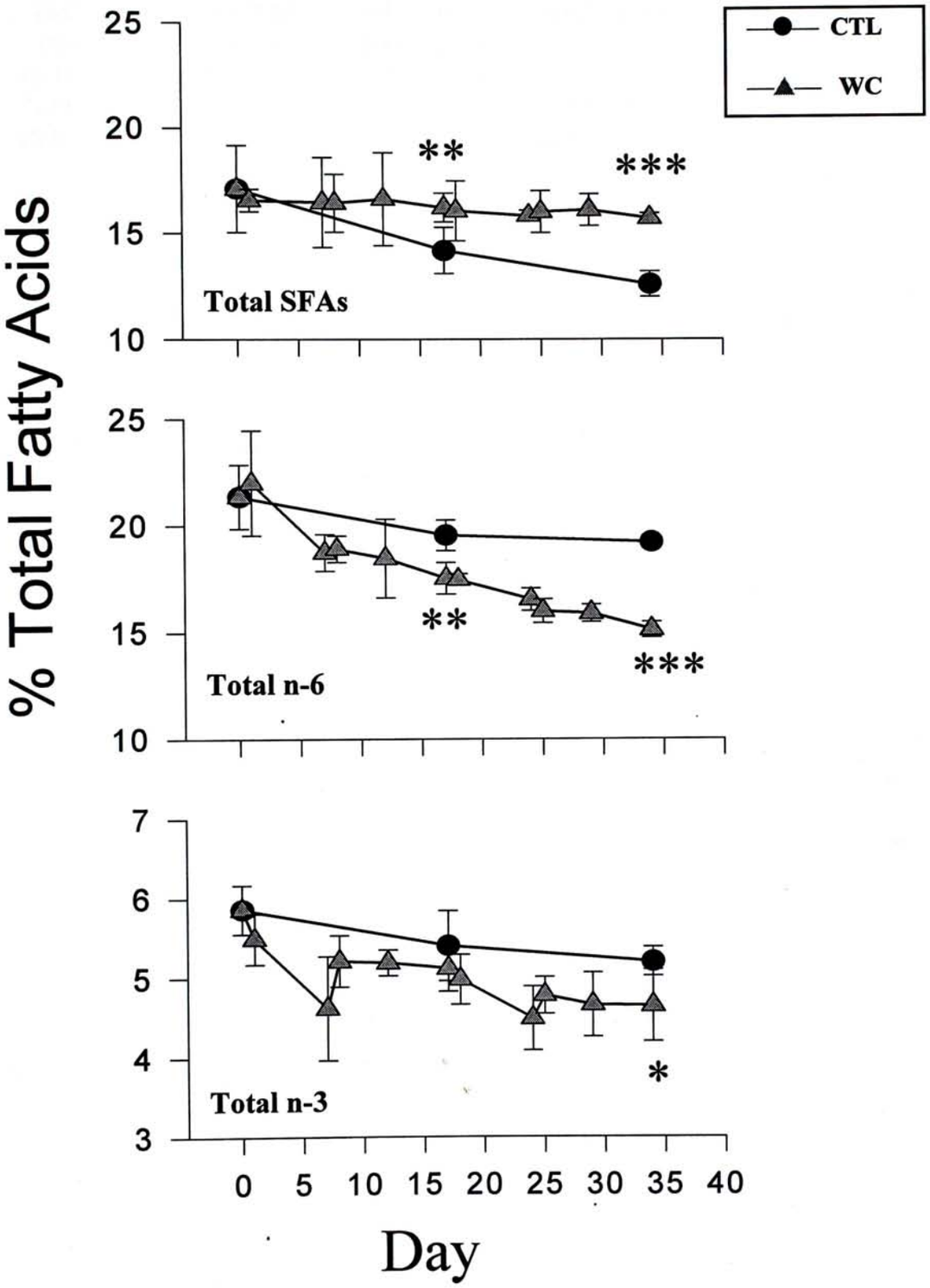


Figure 3.34 Time-course changes in total SFAs, total n-6 and total n-3 in adipose tissue in rats fed MF diet. (*p<0.5, **p<0.01 and ***p<0.001, difference between WC and CTL rats.)

Table 3.7 Fatty Acid Composition of adipose tissue of rats from HF group

Fatty Acids	CTL				
	Day 0	**Day 7**	**Day 17**	**Day 24**	**Day 34**
14:0	0.93 ± 0.08	0.88 ± 0.07	0.67 ± 0.13	0.63 ± 0.01	0.53 ± 0.07
16:0	12.70 ± 2.01	12.96 ± 0.76	11.25 ± 0.99	10.81 ± 0.24	9.72 ± 0.47
16:1n-7	1.88 ± 0.19	1.67 ± 0.29	1.44 ± 0.27	1.43 ± 0.10	1.31 ± 0.09
18:0	2.73 ± 0.21	2.79 ± 0.20	2.44 ± 0.13	2.31 ± 0.06	2.29 ± 0.19
18:1n-9	47.84 ± 0.60	48.15 ± 1.81	52.30 ± 1.93	52.61 ± 0.79	55.17 ± 1.38
18:1n-7	2.45 ± 0.33	2.29 ± 0.09	2.30 ± 0.21	2.50 ± 0.21	2.48 ± 0.07
18:2n-6	20.66 ± 1.16	20.64 ± 0.57	19.65 ± 0.84	18.99 ± 0.40	18.45 ± 0.31
20:3n-6	0.20 ± 0.01	0.19 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.11 ± 0.01
20:4n-6	0.47 ± 0.09	0.41 ± 0.07	0.35 ± 0.03	0.27 ± 0.02	0.29 ± 0.02
22:4n-6	0.07 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.00	0.04 ± 0.01
22:5n-6	0.08 ± 0.01	0.07 ± 0.00	0.07 ± 0.01	0.07 ± 0.00	0.06 ± 0.01
18:3n-3	5.44 ± 0.20	5.24 ± 0.23	5.11 ± 0.54	4.98 ± 0.10	4.88 ± 0.17
20:5n-3	0.11 ± 0.03	0.10 ± 0.03	0.06 ± 0.01	0.08 ± 0.02	0.04 ± 0.01
22:5n-3	0.17 ± 0.04	0.15 ± 0.02	0.25 ± 0.03	0.13 ± 0.03	0.18 ± 0.02
22:6n-3	0.26 ± 0.06	0.20 ± 0.04	0.15 ± 0.02	0.13 ± 0.03	0.06 ± 0.01
Total SFAs	16.78 ± 2.15	17.00 ± 0.91	14.64 ± 1.31	14.07 ± 0.22	12.84 ± 0.73
Total MUFAs	54.04 ± 0.71	54.18 ± 1.62	57.78 ± 1.60	58.06 ± 0.95	60.07 ± 1.26
Total n-6	21.48 ± 1.29	21.35 ± 0.66	20.26 ± 0.90	19.53 ± 0.45	18.82 ± 0.36
Total n-3	5.98 ± 0.34	5.69 ± 0.22	5.58 ± 0.60	5.32 ± 0.17	5.03 ± 0.21

Data are expressed as mean ± SD (n=5).

continued

Fatty Acids	WC 1				
	Day 1	**Day 7**	**Day 8**	**Day 12**	**Day 17**
14:0	0.88 ± 0.04	0.84 ± 0.16	0.87 ± 0.11	0.77 ± 0.13	0.75 ± 0.05
16:0	13.06 ± 0.50	12.86 ± 1.91	12.84 ± 1.10	13.29 ± 1.43	13.32 ± 0.48*
16:1n-7	1.87 ± 0.11	1.71 ± 0.55	1.72 ± 0.31	1.50 ± 0.31	1.50 ± 0.14
18:0	2.39 ± 0.68	2.65 ± 0.12	2.75 ± 0.22	2.61 ± 0.33	2.42 ± 0.11
18:1n-9	48.11 ± 1.47	49.83 ± 1.61	49.86 ± 0.84	52.06 ± 0.84	51.56 ± 1.05
18:1n-7	2.50 ± 0.13	2.70 ± 0.40	2.62 ± 0.32	2.57 ± 0.15	2.58 ± 0.42
18:2n-6	18.78 ± 2.60	17.59 ± 0.98**	17.54 ± 0.66	17.02 ± 1.88	16.79 ± 0.90**
20:3n-6	0.17 ± 0.01*	0.17 ± 0.01	0.18 ± 0.02	0.17 ± 0.01	0.15 ± 0.01
20:4n-6	0.29 ± 0.06*	0.29 ± 0.04	0.28 ± 0.05	0.26 ± 0.06	0.28 ± 0.05
22:4n-6	0.09 ± 0.02	0.03 ± 0.00	0.04 ± 0.01	0.07 ± 0.02	0.04 ± 0.01
22:5n-6	0.07 ± 0.01	0.04 ± 0.00***	0.05 ± 0.01	0.04 ± 0.00	0.06 ± 0.01
18:3n-3	5.32 ± 0.31	4.53 ± 0.38*	4.81 ± 0.34	5.47 ± 0.16	5.42 ± 0.30
20:5n-3	0.09 ± 0.03	0.05 ± 0.00***	0.05 ± 0.01	0.07 ± 0.01	0.08 ± 0.02
22:5n-3	0.13 ± 0.03	0.08 ± 0.01***	0.11 ± 0.02	0.13 ± 0.04	0.12 ± 0.03***
22:6n-3	0.12 ± 0.03***	0.11 ± 0.01***	0.12 ± 0.02	0.12 ± 0.02	0.12 ± 0.01
Total SFAs	16.70 ± 0.51	16.65 ± 2.18	16.80 ± 1.28	16.99 ± 1.84	16.87 ± 0.60
Total MUFAs	54.19 ± 1.39	55.57 ± 0.83	55.74 ± 0.72	57.56 ± 0.66	56.96 ± 1.13
Total n-6	19.41 ± 2.69	18.12 ± 1.04**	18.09 ± 0.74	17.57 ± 1.97	17.32 ± 0.98**
Total n-3	5.66 ± 0.39	4.77 ± 0.30**	5.09 ± 0.39	5.79 ± 0.22	5.72 ± 0.37

Data are expressed as mean ± SD (n=5).
*p<0.05, **p<0.01 and ***p<0.001, between the CTL and WC rats in the corresponding group.

continued

Fatty Acids	WC 2				
	Day 18	**Day 24**	**Day 25**	**Day 29**	**Day 34**
14:0	0.71 ± 0.07	0.71 ± 0.03**	0.66 ± 0.08	0.56 ± 0.03	0.55 ± 0.05
16:0	12.88 ± 0.70	12.22 ± 0.28**	12.54 ± 0.69	12.75 ± 0.54	12.7 ± 0.46***
16:1n-7	1.51 ± 0.15	1.51 ± 0.09	1.59 ± 0.24	1.52 ± 0.10	1.51 ± 0.16
18:0	2.54 ± 0.11	2.57 ± 0.10*	2.47 ± 0.15	2.41 ± 0.03	2.43 ± 0.13
18:1n-9	52.16 ± 0.64	52.51 ± 0.62	53.51 ± 1.32	54.18 ± 0.37	53.88 ± 1.63
18:1n-7	2.79 ± 0.43	2.39 ± 0.15	2.27 ± 0.24	2.28 ± 0.19	2.50 ± 0.42
18:2n-6	15.46 ± 0.27	14.88 ± 0.68***	15.46 ± 0.67	14.36 ± 0.52	14.02 ± 0.51***
20:3n-6	0.14 ± 0.03	0.14 ± 0.02	0.13 ± 0.02	0.12 ± 0.01	0.12 ± 0.02
20:4n-6	0.23 ± 0.05	0.23 ± 0.04	0.23 ± 0.04	0.23 ± 0.02	0.23 ± 0.03*
22:4n-6	0.04 ± 0.01	0.04 ± 0.00	0.04 ± 0.01	0.04 ± 0.00	0.05 ± 0.02
22:5n-6	0.07 ± 0.01	0.07 ± 0.00	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.01
18:3n-3	5.14 ± 0.34	4.23 ± 0.30*	4.82 ± 0.30	4.78 ± 0.44	4.56 ± 0.48
20:5n-3	0.06 ± 0.01	0.04 ± 0.01*	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.02
22:5n-3	0.09 ± 0.01	0.09 ± 0.02*	0.11 ± 0.03	0.08 ± 0.01	0.11 ± 0.04*
22:6n-3	0.09 ± 0.01	0.07 ± 0.04*	0.08 ± 0.05	0.08 ± 0.02	0.08 ± 0.03
Total SFAs	16.80 ± 0.83	15.83 ± 0.23**	16.02 ± 0.86	16.25 ± 0.58	16.75 ± 0.60***
Total MUFAs	58.15 ± 0.46	59.42 ± 0.65	59.77 ± 1.18	59.86 ± 0.24	59.80 ± 1.33
Total n-6	15.93 ± 0.35	15.36 ± 0.76***	15.93 ± 0.75	14.82 ± 0.56	14.49 ± 0.58***
Total n-3	5.38 ± 0.37	4.44 ± 0.46*	5.07 ± 0.39	4.99 ± 0.47	4.81 ± 0.56

Data are expressed as mean ± SD (n=5).
*p<0.05, **p<0.01 and ***p<0.001, between the CTL and WC rats in the corresponding group.

Table 3.8 Fatty Acid Composition of adipose tissue of rats from MF group

Fatty Acids	CTL		
	Day 0	**Day 17**	**Day 34**
14:0	0.94 ± 0.09	0.65 ± 0.11	0.51 ± 0.06
16:0	12.97 ± 1.87	10.80 ± 0.87	9.41 ± 0.40
16:1n-7	1.89 ± 0.18	1.36 ± 0.24	1.04 ± 0.09
18:0	2.78 ± 0.23	2.32 ± 0.15	2.25 ± 0.17
18:1n-9	47.48 ± 1.33	50.73 ± 1.62	53.45 ± 1.17
18:1n-7	2.39 ± 0.28	2.25 ± 0.18	2.41 ± 0.06
18:2n-6	20.58 ± 1.54	19.06 ± 0.71	18.85 ± 0.27
20:3n-6	0.20 ± 0.01	0.14 ± 0.01	0.13 ± 0.02
20:4n-6	0.44 ± 0.10	0.27 ± 0.02	0.17 ± 0.02
22:4n-6	0.07 ± 0.01	0.04 ± 0.01	0.04 ± 0.00
22:5n-6	0.07 ± 0.01	0.07 ± 0.00	0.06 ± 0.00
18:3n-3	5.31 ± 0.22	5.08 ± 0.45	4.95 ± 0.15
20:5n-3	0.13 ± 0.03	0.06 ± 0.01	0.04 ± 0.01
22:5n-3	0.17 ± 0.04	0.14 ± 0.03	0.11 ± 0.05
22:6n-3	0.24 ± 0.07	0.11 ± 0.02	0.10 ± 0.03
Total SFAs	17.11 ± 2.06	14.19 ± 1.10	12.59 ± 0.60
Total MUFAs	52.39 ± 1.39	54.98 ± 1.35	57.48 ± 1.06
Total n-6	21.36 ± 1.50	19.57 ± 0.72	19.24 ± 0.29
Total n-3	5.86 ± 0.31	5.40 ± 0.44	5.20 ± 0.18

Data are expressed as mean ± SD (n=5).

continued

Fatty Acids	WC 1				
	Day 1	**Day 7**	**Day 8**	**Day 12**	**Day 17**
14:0	0.85 ± 0.04	0.82 ± 0.16	0.84 ± 0.11	0.75 ± 0.13	0.67 ± 0.05
16:0	12.66 ± 0.48	12.48 ± 1.85	12.46 ± 1.07	12.90 ± 1.51	12.90 ± 0.57**
16:1n-7	1.81 ± 0.11	1.66 ± 0.53	1.67 ± 0.30	1.36 ± 0.30	1.36 ± 0.14
18:0	2.52 ± 0.25	2.57 ± 0.12	2.67 ± 0.21	2.53 ± 0.32	2.34 ± 0.11
18:1n-9	46.67 ± 1.43	48.33 ± 1.56	48.36 ± 0.82	50.50 ± 0.82	50.01 ± 1.02
18:1n-7	2.43 ± 0.13	2.62 ± 0.39	2.54 ± 0.31	2.49 ± 0.14	2.50 ± 0.41
18:2n-6	21.43 ± 2.52	18.22 ± 0.91*	18.40 ± 0.61	17.95 ± 1.82	17.03 ± 0.76***
20:3n-6	0.17 ± 0.01	0.17 ± 0.01*	0.17 ± 0.02	0.17 ± 0.01	0.15 ± 0.01
20:4n-6	0.28 ± 0.06	0.28 ± 0.04**	0.27 ± 0.05	0.25 ± 0.03	0.27 ± 0.04
22:4n-6	0.06 ± 0.01	0.03 ± 0.00***	0.04 ± 0.01	0.06 ± 0.01	0.04 ± 0.01
22:5n-6	0.07 ± 0.00	0.04 ± 0.00***	0.04 ± 0.00	0.04 ± 0.00	0.06 ± 0.01
18:3n-3	5.16 ± 0.30	4.37 ± 0.65*	4.95 ± 0.33	4.92 ± 0.14	4.87 ± 0.27
20:5n-3	0.08 ± 0.03	0.05 ± 0.00***	0.05 ± 0.01	0.06 ± 0.01	0.07 ± 0.02
22:5n-3	0.13 ± 0.03	0.12 ± 0.02	0.11 ± 0.02	0.14 ± 0.02	0.11 ± 0.03
22:6n-3	0.12 ± 0.03	0.08 ± 0.01	0.09 ± 0.02	0.07 ± 0.04	0.06 ± 0.03
Total SFAs	16.56 ± 0.53	16.47 ± 2.14	16.44 ± 1.37	16.63 ± 2.20	16.22 ± 0.69**
Total MUFAs	51.48 ± 1.35	53.24 ± 0.81	53.20 ± 0.69	54.95 ± 0.64	54.45 ± 1.10
Total n-6	22.01 ± 2.46	18.74 ± 0.87**	18.92 ± 0.62	18.48 ± 1.85	17.54 ± 0.74**
Total n-3	5.49 ± 0.32	4.62 ± 0.65*	5.21 ± 0.32	5.19 ± 0.16	5.12 ± 0.29

Data are expressed as mean ± SD (n=5).
*p<0.05, **p<0.01 and ***p<0.001, between the CTL and WC rats in the corresponding group.

continued

Fatty Acids	WC 2				
	Day 18	**Day 24**	**Day 25**	**Day 29**	**Day 34**
14:0	0.69 ± 0.07	0.69 ± 0.02	0.64 ± 0.08	0.54 ± 0.03	0.53 ± 0.05
16:0	12.45 ± 1.23	12.34 ± 0.31	12.67 ± 0.83	12.90 ± 0.71	12.49 ± 0.21***
16:1n-7	1.35 ± 0.15	1.31 ± 0.08	1.25 ± 0.23	1.15 ± 0.11	1.15 ± 0.17
18:0	2.46 ± 0.11	2.49 ± 0.10	2.39 ± 0.15	2.34 ± 0.03	2.36 ± 0.12
18:1n-9	50.60 ± 0.62	50.93 ± 0.60	51.91 ± 1.28	52.56 ± 0.36	52.26 ± 1.59
18:1n-7	2.71 ± 0.42	2.32 ± 0.15	2.20 ± 0.23	2.21 ± 0.19	2.43 ± 0.41
18:2n-6	17.01 ± 0.23	16.17 ± 0.55	15.58 ± 0.54	15.53 ± 0.41	14.75 ± 0.38***
20:3n-6	0.14 ± 0.02	0.15 ± 0.02	0.16 ± 0.02	0.14 ± 0.01	0.16 ± 0.02
20:4n-6	0.22 ± 0.05	0.12 ± 0.02	0.14 ± 0.02	0.12 ± 0.01	0.12 ± 0.02**
22:4n-6	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.01	0.04 ± 0.00	0.04 ± 0.00
22:5n-6	0.07 ± 0.00	0.07 ± 0.00	0.07 ± 0.01	0.07 ± 0.00	0.06 ± 0.01
18:3n-3	4.78 ± 0.32	4.33 ± 0.38	4.61 ± 0.27	4.48 ± 0.39	4.47 ± 0.43*
20:5n-3	0.05 ± 0.00	0.04 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.02
22:5n-3	0.08 ± 0.00	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.07 ± 0.02
22:6n-3	0.06 ± 0.01	0.07 ± 0.02	0.06 ± 0.03	0.06 ± 0.02	0.06 ± 0.03
Total SFAs	16.07 ± 1.42	15.84 ± 0.26	16.03 ± 0.99	16.12 ± 0.75	15.72 ± 0.24***
Total MUFAs	55.28 ± 0.45	55.20 ± 0.63	56.00 ± 1.15	56.50 ± 0.23	56.41 ± 1.28
Total n-6	17.48 ± 0.27	16.55 ± 0.53	16.00 ± 0.56	15.90 ± 0.41	15.13 ± 0.36***
Total n-3	4.98 ± 0.31	4.49 ± 0.40	4.78 ± 0.23	4.66 ± 0.40	4.65 ± 0.45*

Data are expressed as mean ± SD (n=5).
*p<0.05, **p<0.01 and ***p<0.001, between the CTL and WC rats in the corresponding group.

3.4.7.3 Liver Fatty Acids

3.4.7.3.1 Liver Total Lipids

The fatty acid composition of liver total lipids in WC rats showed no significant difference between the CTL and WC rats fed both HF and MF diet at the end of the cycles (Tables 3.9 and 3.10). However, WC rats in the HF group had a considerably lower proportion of 18:1n-9 and 22:5n-6, while they had a substantially higher proportion of 18:2n-6, 18:3n-3 and 22:4n-6 compared with the CTL rats in the second food restriction period. Furthermore, in the first food restriction, 16:0 and 16:1n-7 were significantly decreased but 18:2n-6 and 18:3n-3 were greatly increased in the WC rats fed MF diet compared with the controls.

3.4.7.3.2 Liver PL

Fatty acid composition of liver PL in WC rats and CTL rats changed similarly (Tables 3.11 and 3.12). There was no significant difference in the fatty acid composition of liver PL between the WC and CTL rats at the end of the 2 cycles. However, 20:3n-6 was extensively reduced in WC rats fed HF diet after 2 weight cycles. During the first food restriction, both 18:1n-9 and vaccenic acid (18:1n-7) were markedly decreased in the WC rats of the HF group, while only 18:1n-9 was significantly reduced in the WC rats during the second food restriction. In MF group, WC rats showed a considerably lower 18:1n-7 but a higher 22:4n-6 during the first food restriction.

Table 3.9 Fatty Acid Composition of liver total lipids of rats from HF group

Fatty Acids	CTL				
	Day 0	**Day 7**	**Day 17**	**Day 24**	**Day 34**
14:0	0.62 ± 0.10	0.63 ± 0.11	0.57 ± 0.07	0.61 ± 0.12	0.62 ± 0.09
16:0	21.31 ± 2.07	20.00 ± 2.84	20.00 ± 1.23	18.29 ± 1.27	18.90 ± 1.87
16:1n-7	3.55 ± 0.85	3.14 ± 0.57	2.83 ± 0.41	2.94 ± 0.51	2.48 ± 0.48
18:0	11.44 ± 1.65	11.28 ± 1.65	10.81 ± 1.65	11.19 ± 0.62	11.47 ± 1.80
18:1n-9	19.55 ± 0.70	19.74 ± 1.13	21.63 ± 1.52	22.99 ± 2.50	24.59 ± 3.85
18:1n-7	5.40 ± 0.90	5.47 ± 0.77	5.14 ± 0.49	4.92 ± 0.47	4.46 ± 0.61
18:2n-6	10.05 ± 1.34	10.03 ± 1.81	9.29 ± 1.62	9.45 ± 1.25	8.43 ± 1.56
20:3n-6	0.81 ± 0.20	0.75 ± 0.14	0.60 ± 0.08	0.58 ± 0.04	0.45 ± 0.13
20:4n-6	10.94 ± 1.29	11.00 ± 1.86	11.97 ± 0.44	11.80 ± 1.37	12.45 ± 1.74
22:4n-6	0.21 ± 0.05	0.19 ± 0.03	0.17 ± 0.02	0.17 ± 0.02	0.16 ± 0.04
22:5n-6	0.19 ± 0.02	0.19 ± 0.02	0.15 ± 0.04	0.45 ± 0.13	0.10 ± 0.02
18:3n-3	0.58 ± 0.13	0.62 ± 0.08	0.69 ± 0.10	0.86 ± 0.13	0.86 ± 0.20
20:5n-3	0.76 ± 0.13	0.71 ± 0.08	0.60 ± 0.17	0.58 ± 0.15	0.59 ± 0.13
22:5n-3	0.67 ± 0.16	0.65 ± 0.11	0.65 ± 0.11	0.63 ± 0.20	0.61 ± 0.07
22:6n-3	3.74 ± 0.82	3.68 ± 0.44	3.87 ± 0.38	3.79 ± 0.37	3.52 ± 0.89
Total SFAs	33.89 ± 3.53	33.66 3.51	32.59 ± 2.05	30.87 ± 1.60	31.77 ± 1.51
Total MUFAs	29.20 ± 1.71	29.71 ± 0.95	30.54 ± 1.87	31.94 ± 2.52	32.46 ± 3.94
Total n-6	22.21 ± 2.55	22.16 ± 3.24	22.17 ± 1.64	22.45 ± 2.25	21.59 ± 2.51
Total n-3	5.76 ± 0.82	5.67 ± 0.59	5.80 ± 0.62	5.86 ± 0.60	5.57 ± 1.14
Total FAs (mg/g)	36.40 ± 3.65	37.02 ± 4.34	41.15 ± 6.81	38.12 ± 4.93	38.13 ± 5.77

Data are expressed as mean ± SD (n=5).

continued

Fatty Acids	WC 1				
	Day 1	**Day 7**	**Day 8**	**Day 12**	**Day 17**
14:0	0.61 ± 0.10	0.62 ± 0.20	0.57 ± 0.15	0.63 ± 0.09	0.63 ± 0.22
16:0	21.69 ± 1.75	20.93 ± 3.09	21.59 ± 2.52	20.92 ± 0.84	20.03 ± 2.42
16:1n-7	3.44 ± 0.54	2.58 ± 0.25	2.68 ± 0.50	2.73 ± 0.53	3.05 ± 0.67
18:0	11.77 ± 1.93	11.77 ± 1.56	12.12 ± 1.23	11.05 ± 2.09	11.74 ± 2.10
18:1n-9	19.77 ± 3.80	18.38 ± 1.72	19.39 ± 3.09	20.22 ± 1.70	20.57 ± 2.49
18:1n-7	5.38 ± 0.99	4.91 ± 0.26	4.69 ± 0.36	5.13 ± 0.51	5.21 ± 0.62
18:2n-6	10.42 ± 2.01	13.25 ± 1.52	10.94 ± 2.17	9.98 ± 1.11	9.63 ± 1.39
20:3n-6	0.76 ± 0.11	0.73 ± 0.12	0.69 ± 0.11	0.62 ± 0.12	0.62 ± 0.12
20:4n-6	10.57 ± 1.90	11.29 ± 2.42	11.63 ± 2.09	12.35 ± 2.61	12.20 ± 1.32
22:4n-6	0.20 ± 0.03	0.16 ± 0.02	0.20 ± 0.03	0.17 ± 0.03	0.20 ± 0.03
22:5n-6	0.19 ± 0.10	0.22 ± 0.03	0.18 ± 0.03	0.15 ± 0.02	0.17 ± 0.07
18:3n-3	0.63 ± 0.15	0.90 ± 0.13	0.92 ± 0.20	0.78 ± 0.18	0.76 ± 0.07
20:5n-3	0.61 ± 0.19	0.60 ± 0.12	0.67 ± 0.16	0.61 ± 0.05	0.62 ± 0.19
22:5n-3	0.66 ± 0.15	0.60 ± 0.08	0.64 ± 0.11	0.59 ± 0.07	0.63 ± 0.06
22:6n-3	3.39 ± 1.29	3.53 ± 0.71	3.19 ± 0.77	3.92 ± 0.63	3.91 ± 1.14
Total SFAs	34.82 ± 2.70	34.25 ± 2.51	35.04 ± 3.16	33.30 ± 1.44	33.05 ± 1.59
Total MUFAs	29.47 ± 3.06	26.88 ± 1.73	27.41 ± 3.15	29.05 ± 1.31	29.78 ± 2.83
Total n-6	22.14 ± 2.68	25.46 ± 2.67	23.64 ± 2.86	23.26 ± 1.88	22.82 ± 2.05
Total n-3	5.29 ± 1.29	5.82 ± 0.80	5.41 ± 0.74	5.90 ± 0.79	5.91 ± 1.30
Total FAs (mg/g)	36.74 ± 3.45	32.72 ± 3.07	35.56 ± 3.12	37.97 ± 1.19	39.26 ± 2.22

Data are expressed as mean ± SD (n=5).

continued

Fatty Aicds	WC 2				
	Day 18	**Day 24**	**Day 25**	**Day 29**	**Day 34**
14:0	0.71 ± 0.07	0.61 ± 0.15	0.65 ± 0.22	0.69 ± 0.19	0.64 ± 0.15
16:0	18.97 ± 2.36	20.15 ± 2.52	20.68 ± 1.35	19.77 ± 3.09	19.49 ± 2.47
16:1n-7	2.74 ± 0.38	2.55 ± 0.24	2.94 ± 1.23	2.96 ± 0.84	2.69 ± 0.60
18:0	12.41 ± 2.00	12.05 ± 1.62	12.49 ± 0.86	11.58 ± 0.48	11.76 ± 1.57
18:1n-9	20.95 ± 2.13	17.14 ± 2.47**	18.68 ± 2.86	22.04 ± 4.49	23.81 ± 2.76
18:1n-7	5.48 ± 0.83	4.94 ± 0.32	4.53 ± 0.53	4.54 ± 0.51	4.70 ± 0.80
18:2n-6	10.22 ± 1.63	13.25 ± 1.23**	10.13 ± 2.21	8.80 ± 1.49	8.57 ± 0.97
20:3n-6	0.70 ± 0.10	0.65 ± 0.07	0.65 ± 0.10	0.57 ± 0.12	0.67 ± 0.15
20:4n-6	11.44 ± 1.49	11.92 ± 2.21	12.51 ± 1.46	12.36 ± 2.36	11.54 ± 2.58
22:4n-6	0.19 ± 0.04	0.23 ± 0.02**	0.23 ± 0.02	0.15 ± 0.04	0.17 ± 0.03
22:5n-6	0.17 ± 0.03	0.16 ± 0.05**	0.15 ± 0.01	0.16 ± 0.04	0.16 ± 0.05
18:3n-3	0.85 ± 0.09	1.29 ± 0.22**	1.02 ± 0.18	0.86 ± 0.20	0.81 ± 0.14
20:5n-3	0.67 ± 0.21	0.60 ± 0.22	0.62 ± 0.12	0.59 ± 0.14	0.61 ± 0.16
22:5n-3	0.65 ± 0.18	0.69 ± 0.11	0.62 ± 0.14	0.60 ± 0.09	0.62 ± 0.13
22:6n-3	3.72 ± 0.68	3.95 ± 0.78	3.97 ± 0.89	3.77 ± 1.16	3.47 ± 1.13
Total SFAs	32.92 ± 3.46	33.68 ± 2.29	34.71 ± 1.43	32.76 ± 3.28	32.60 ± 2.84
Total MUFAs	29.89 ± 1.85	25.53 ± 2.35**	26.89 ± 3.55	30.60 ± 4.72	32.15 ± 2.72
Total n-6	22.72 ± 3.03	26.21 ± 2.18	23.67 ± 2.51	22.05 ± 2.85	21.10 ± 2.31
Total n-3	5.88 ± 0.73	6.53 ± 0.99	6.22 ± 0.75	5.82 ± 1.23	5.50 ± 1.26
Total FAs (mg/g)	35.37 ± 3.63	35.67 ± 4.38	41.93 ± 7.92	39.76 ± 8.57	35.51 ± 4.26

Data are expressed as mean ± SD (n=5).
*p<0.05, **p<0.01 and ***p<0.001, between the CTL and WC rats in the corresponding group.

Table 3.10 Fatty Acid Composition of liver total lipids of rats from MF group

Fatty Acids	CTL		
	Day 0	Day 17	Day 34
14:0	0.58 ± 0.07	0.50 ± 0.08	0.52 ± 0.13
16:0	22.19 ± 1.68	18.90 ± 1.16	18.67 ± 1.59
16:1n-7	3.34 ± 0.87	2.08 ± 0.64	1.94 ± 0.71
18:0	10.77 ± 0.61	11.27 ± 0.65	11.57 ± 1.46
18:1n-9	20.07 ± 0.97	23.29 ± 1.08	25.12 ± 2.45
18:1n-7	5.17 ± 0.64	4.55 ± 0.34	4.08 ± 0.28
18:2n-6	9.62 ± 2.34	8.77 ± 1.27	7.94 ± 1.39
20:3n-6	0.74 ± 0.13	0.56 ± 0.07	0.43 ± 0.14
20:4n-6	10.45 ± 1.35	12.33 ± 1.10	13.36 ± 1.17
22:4n-6	0.19 ± 0.03	0.17 ± 0.02	0.15 ± 0.04
22:5n-6	0.19 ± 0.01	0.15 ± 0.05	0.11 ± 0.03
18:3n-3	0.52 ± 0.20	0.76 ± 0.14	0.85 ± 0.19
20:5n-3	0.71 ± 0.05	0.60 ± 0.18	0.59 ± 0.12
22:5n-3	0.61 ± 0.14	0.59 ± 0.11	0.61 ± 0.02
22:6n-3	3.60 ± 0.50	3.80 ± 0.35	3.52 ± 0.56
Total SFAs	34.30 ± 2.07	32.05 ± 2.16	31.71 ± 0.63
Total MUFAs	29.52 ± 1.54	31.01 ± 1.90	31.99 ± 2.25
Total n-6	21.19 ± 3.54	21.98 ± 1.83	21.99 ± 1.73
Total n-3	5.45 ± 0.45	5.76 ± 0.59	5.58 ± 0.75
Total FAs (mg/g)	36.02 ± 2.82	39.37 ± 7.19	35.43 ± 2.18

Data are expressed as mean ± SD (n=5).

continued

Fatty Acids	WC 1				
	Day 1	**Day 7**	**Day 8**	**Day 12**	**Day 17**
14:0	0.57 ± 0.10	0.54 ± 0.16	0.49 ± 0.21	0.69 ± 0.10	0.58 ± 0.22
16:0	22.17 ± 1.21	15.84 ± 0.85***	19.70 ± 2.48	18.10 ± 3.13	18.57 ± 0.64
16:1n-7	3.20 ± 0.62	1.42 ± 1.14*	2.49 ± 0.39	2.56 ± 0.55	1.93 ± 0.04
18:0	11.46 ± 1.27	12.00 ± 2.04	10.85 ± 0.33	11.06 ± 1.39	11.45 ± 0.64
18:1n-9	18.71 ± 6.51	22.52 ± 1.98	22.70 ± 0.88	23.68 ± 1.44	23.41 ± 0.33
18:1n-7	5.55 ± 2.24	4.55 ± 0.23	4.35 ± 0.12	5.00 ± 0.17	4.19 ± 0.62
18:2n-6	9.00 ± 1.91	13.18 ± 1.06*	11.06 ± 1.03	9.39 ± 0.63	8.66 ± 0.41
20:3n-6	0.74 ± 0.03	0.72 ± 0.19	0.64 ± 0.11	0.60 ± 0.11	0.58 ± 0.02
20:4n-6	11.59 ± 2.44	11.47 ± 1.24	10.68 ± 1.10	11.87 ± 1.04	13.56 ± 0.50
22:4n-6	0.20 ± 0.02	0.13 ± 0.04	0.18 ± 0.04	0.19 ± 0.04	0.17 ± 0.02
22:5n-6	0.21 ± 0.13	0.22 ± 0.03	0.17 ± 0.02	0.16 ± 0.08	0.14 ± 0.02
18:3n-3	0.60 ± 0.15	1.17 ± 0.27**	1.33 ± 0.08	0.91 ± 0.17	0.64 ± 0.01
20:5n-3	0.60 ± 0.21	0.61 ± 0.17	0.60 ± 0.12	0.60 ± 0.17	0.57 ± 0.02
22:5n-3	0.64 ± 0.14	0.59 ± 0.09	0.57 ± 0.08	0.60 ± 0.04	0.55 ± 0.02
22:6n-3	3.26 ± 0.88	3.48 ± 0.34	2.90 ± 0.64	3.70 ± 0.87	3.62 ± 0.28
Total SFAs	34.94 ± 2.17	29.32 ± 1.30**	31.82 ± 2.49	30.63 ± 2.31	31.31 ± 0.97
Total MUFAs	28.45 ± 4.20	29.14 ± 1.42	30.30 ± 0.91	32.22 ± 1.41	30.48 ± 0.45
Total n-6	21.73 ± 1.79	25.72 ± 1.20	22.73 ± 1.68	22.07 ± 1.10	23.10 ± 0.40
Total n-3	5.10 ± 0.83	5.85 ± 0.50	5.40 ± 0.71	5.95 ± 1.02	5.37 ± 0.29
Total FAs (mg/g)	34.61 ± 3.62	32.35 ± 2.72	31.26 ± 1.94	33.34 ± 2.26	36.98 ± 2.17

Data are expressed as mean ± SD (n=5).
*p<0.05, **p<0.01 and ***p<0.001, between the CTL and WC rats in the corresponding group.

Continued

Fatty Acids	WC 2				
	Day 18	**Day 24**	**Day 25**	**Day 29**	**Day 34**
14:0	0.69 ± 0.03	0.59 ± 0.22	0.82 ± 0.21	0.82 ± 0.18	0.64 ± 0.14
16:0	17.76 ± 2.27	18.20 ± 2.02	20.50 ± 1.72	19.42 ± 3.04	18.25 ± 5.24
16:1n-7	1.71 ± 0.21	1.35 ± 0.33	2.74 ± 1.16	2.86 ± 0.71	2.52 ± 0.62
18:0	11.38 ± 0.73	12.22 ± 0.66	11.95 ± 0.51	11.76 ± 1.54	13.34 ± 3.48
18:1n-9	25.04 ± 1.38	21.97 ± 1.27	20.83 ± 2.54	21.59 ± 3.93	20.36 ± 6.07
18:1n-7	4.69 ± 0.48	4.15 ± 0.20	4.27 ± 0.27	4.04 ± 0.28	4.68 ± 1.25
18:2n-6	9.64 ± 1.20	10.64 ± 0.76	8.62 ± 1.77	8.69 ± 1.41	8.42 ± 2.20
20:3n-6	0.65 ± 0.13	0.66 ± 0.15	0.63 ± 0.06	0.56 ± 0.10	0.70 ± 0.12*
20:4n-6	11.23 ± 1.77	12.20 ± 0.59	12.15 ± 0.63	12.57 ± 1.45	13.22 ± 4.60
22:4n-6	0.18 ± 0.04	0.23 ± 0.04	0.20 ± 0.02	0.15 ± 0.04	0.17 ± 0.08
22:5n-6	0.16 ± 0.03	0.17 ± 0.07	0.15 ± 0.01	0.16 ± 0.04	0.17 ± 0.07
18:3n-3	0.82 ± 0.10	1.07 ± 0.19	0.64 ± 0.28	0.74 ± 0.30	0.74 ± 0.24
20:5n-3	0.61 ± 0.21	0.67 ± 0.35	0.61 ± 0.13	0.60 ± 0.15	0.71 ± 0.36
22:5n-3	0.73 ± 0.04	0.63 ± 0.12	0.62 ± 0.15	0.61 ± 0.11	0.68 ± 0.22
22:6n-3	3.36 ± 0.23	3.96 ± 0.67	3.79 ± 0.59	3.74 ± 0.77	3.67 ± 0.99
Total SFAs	30.54 ± 2.84	31.75 ± 1.39	34.05 ± 2.09	32.70 ± 3.53	33.09 ± 5.05
Total MUFAs	32.22 ± 1.28	28.19 ± 1.23	28.70 ± 3.24	29.62 ± 3.84	28.62 ± 4.69
Total n-6	21.86 ± 2.54	23.89 ± 0.49	21.75 ± 2.23	22.13 ± 1.54	22.68 ± 6.80
Total n-3	5.52 ± 0.27	6.32 ± 0.97	5.66 ± 0.63	5.69 ± 0.92	5.81 ± 1.56
Total FAs (mg/g)	32.05 ± 3.54	32.32 ± 11.16	35.20 ± 1.14	37.00 ± 8.98	34.01 ± 5.88

Data are expressed as mean ± SD (n=5).
*$p<0.05$, between the CTL and WC rats in the corresponding group.

Table 3.11 Fatty Acid Composition of liver PL of rats from HF group

Fatty Acids	CTL				
	Day 0	**Day 7**	**Day 17**	**Day 24**	**Day 34**
14:0	0.13 ± 0.03	0.14 ± 0.05	0.14 ± 0.02	0.13 ± 0.03	0.15 ± 0.04
16:0	14.16 ± 2.64	14.19 ± 2.70	13.92 ± 1.99	13.80 ± 1.88	13.40 ± 2.18
16:1n-7	0.52 ± 0.13	0.48 ± 0.15	0.49 ± 0.09	0.50 ± 0.09	0.53 ± 0.17
18:0	21.67 ± 3.08	21.71 ± 3.08	20.67 ± 2.44	21.66 ± 1.17	22.27 ± 3.76
18:1n-9	8.91 ± 0.76	8.51 ± 1.71	8.65 ± 0.85	8.57 ± 1.26	8.51 ± 1.19
18:1n-7	4.95 ± 1.00	5.15 ± 0.94	5.05 ± 0.54	5.01 ± 0.58	4.72 ± 1.00
18:2n-6	11.72 ± 1.75	11.54 ± 1.85	10.88 ± 1.95	10.85 ± 3.01	10.07 ± 2.11
20:3n-6	1.33 ± 0.39	1.29 ± 0.32	1.01 ± 0.10	0.99 ± 0.08	0.80 ± 0.27
20:4n-6	22.77 ± 3.63	23.21 ± 3.94	25.38 ± 2.24	24.87 ± 3.16	26.54 ± 4.65
22:4n-6	0.29 ± 0.07	0.28 ± 0.05	0.20 ± 0.03	0.22 ± 0.05	0.21 ± 0.07
22:5n-6	0.33 ± 0.04	0.34 ± 0.05	0.25 ± 0.10	0.25 ± 0.10	0.16 ± 0.06
18:3n-3	0.36 ± 0.08	0.34 ± 0.09	0.35 ± 0.06	0.37 ± 0.08	0.39 ± 0.06
20:5n-3	1.37 ± 0.26	1.35 ± 0.14	1.16 ± 0.39	1.13 ± 0.32	1.17 ± 0.33
22:5n-3	1.06 ± 0.25	1.02 ± 0.20	1.04 ± 0.24	1.08 ± 0.39	1.10 ± 0.21
22:6n-3	6.99 ± 1.41	7.21 ± 0.89	7.73 ± 1.06	7.62 ± 0.72	7.27 ± 2.15
Total SFAs	36.41 ± 4.93	37.10 ± 3.44	36.06 ± 2.33	36.61 ± 2.05	36.85 ± 2.81
Total MUFAs	15.12 ± 0.89	15.47 ± 1.18	15.22 ± 1.35	15.39 ± 1.54	14.99 ± 1.76
Total n-6	36.43 ± 5.03	36.66 ± 4.88	37.72 ± 3.71	37.17 ± 5.36	37.78 ± 6.11
Total n-3	9.78 ± 1.22	9.92 ± 1.04	10.27 ± 1.65	10.20 ± 1.17	9.93 ± 2.66
Total FAs (mg/g)	21.73 ± 1.13	22.05 ± 1.32	25.84 ± 6.73	22.71 ± 1.65	22.35 ± 1.91

Data are expressed as mean ± SD (n=5).

continued

Fatty Acids	WC 1				
	Day 1	**Day 7**	**Day 8**	**Day 12**	**Day 17**
14:0	0.12 ± 0.04	0.12 ± 0.04	0.13 ± 0.05	0.15 ± 0.05	0.16 ± 0.05
16:0	14.07 ± 2.32	14.55 ± 2.14	16.55 ± 3.21	15.57 ± 2.74	13.08 ± 2.13
16:1n-7	0.45 ± 0.07	0.40 ± 0.07	0.44 ± 0.24	0.45 ± 0.06	0.51 ± 0.08
18:0	21.45 ± 3.61	21.09 ± 2.94	23.10 ± 3.21	20.45 ± 4.99	22.44 ± 6.06
18:1n-9	7.42 ± 1.84	5.91 ± 1.39*	7.05 ± 0.78	7.70 ± 1.04	7.58 ± 1.48
18:1n-7	4.56 ± 0.41	3.82 ± 0.31*	4.36 ± 0.57	4.79 ± 1.25	5.03 ± 0.98
18:2n-6	11.29 ± 2.63	12.07 ± 3.32	11.17 ± 2.73	11.27 ± 1.81	10.98 ± 1.99
20:3n-6	1.22 ± 0.29	1.13 ± 0.21	1.16 ± 0.25	1.06 ± 0.19	1.08 ± 0.26
20:4n-6	21.07 ± 3.82	22.04 ± 4.55	23.86 ± 5.69	24.96 ± 4.75	25.58 ± 4.60
22:4n-6	0.25 ± 0.05	0.17 ± 0.03	0.29 ± 0.05	0.21 ± 0.04	0.27 ± 0.05
22:5n-6	0.31 ± 0.18	0.37 ± 0.06	0.32 ± 0.07	0.24 ± 0.05	0.29 ± 0.14
18:3n-3	0.32 ± 0.03	0.37 ± 0.10	0.45 ± 0.10	0.32 ± 0.05	0.46 ± 0.15
20:5n-3	0.99 ± 0.34	1.02 ± 0.28	1.21 ± 0.40	1.10 ± 0.12	1.17 ± 0.40
22:5n-3	1.00 ± 0.25	0.87 ± 0.16	1.01 ± 0.28	0.96 ± 0.17	1.03 ± 0.14
22:6n-3	6.27 ± 2.63	6.45 ± 1.56	6.05 ± 1.75	7.53 ± 1.42	7.69 ± 2.63
Total SFAs	36.64 ± 1.70	36.75 ± 2.99	40.66 ± 5.74	36.96 ± 7.29	36.38 ± 4.71
Total MUFAs	13.45 ± 2.22	11.15 ± 1.55**	12.60 ± 1.20	13.92 ± 1.05	13.82 ± 1.75
Total n-6	34.14 ± 4.85	35.41 ± 2.10	36.80 ± 6.81	37.74 ± 4.29	38.20 ± 5.75
Total n-3	8.58 ± 2.78	9.08 ± 1.69	8.72 ± 2.23	9.91 ± 1.73	10.35 ± 3.02
Total FAs (mg/g)	21.93 ± 2.55	17.48 ± 2.90	19.79 ± 3.22	22.42 ± 1.31	23.44 ± 2.32

Data are expressed as mean ± SD (n=5).
*$p<0.05$ and **$p<0.01$, between the CTL and WC rats in the corresponding group.

continued

Fatty Acids	WC 2				
	Day 18	**Day 24**	**Day 25**	**Day 29**	**Day 34**
14:0	0.14 ± 0.04	0.11 ± 0.03	0.14 ± 0.04	0.13 ± 0.04	0.14 ± 0.03
16:0	13.58 ± 3.02	13.11 ± 2.74	12.40 ± 1.09	12.53 ± 1.23	13.27 ± 2.05
16:1n-7	0.49 ± 0.13	0.42 ± 0.07	0.43 ± 0.16	0.47 ± 0.09	0.46 ± 0.10
18:0	22.95 ± 2.75	20.95 ± 2.01	23.91 ± 2.49	22.40 ± 0.41	22.68 ± 2.36
18:1n-9	7.40 ± 1.57	5.26 ± 0.97**	7.76 ± 1.37	7.51 ± 1.01	8.71 ± 1.53
18:1n-7	5.43 ± 1.95	4.46 ± 0.47	4.14 ± 0.87	3.99 ± 0.57	4.89 ± 0.88
18:2n-6	10.93 ± 2.51	11.18 ± 1.56	10.45 ± 1.31	9.71 ± 1.67	10.28 ± 0.47
20:3n-6	1.20 ± 0.23	1.05 ± 0.10	1.19 ± 0.22	1.04 ± 0.25	1.27 ± 0.35
20:4n-6	22.97 ± 3.70	22.51 ± 4.45	25.80 ± 4.00	25.76 ± 4.93	24.69 ± 6.58
22:4n-6	0.23 ± 0.05	0.30 ± 0.04	0.32 ± 0.03	0.21 ± 0.06	0.22 ± 0.04
22:5n-6	0.30 ± 0.05	0.25 ± 0.09	0.25 ± 0.03	0.27 ± 0.09	0.28 ± 0.13
18:3n-3	0.45 ± 0.05	0.37 ± 0.10	0.34 ± 0.07	0.29 ± 0.05	0.37 ± 0.07
20:5n-3	1.21 ± 0.44	1.04 ± 0.33	1.16 ± 0.27	1.12 ± 0.32	1.17 ± 0.32
22:5n-3	1.11 ± 0.39	1.10 ± 0.19	1.12 ± 0.27	1.11 ± 0.16	1.22 ± 0.37
22:6n-3	7.16 ± 1.55	7.14 ± 1.37	7.99 ± 2.10	7.68 ± 2.45	7.24 ± 2.66
Total SFAs	37.71 ± 4.91	35.05 ± 2.87	37.42 ± 2.19	36.05 ± 1.12	36.99 ± 3.17
Total MUFAs	14.19 ± 0.76	11.12 ± 0.85***	13.04 ± 1.60	12.87 ± 0.97	14.87 ± 0.98
Total n-6	35.63 ± 6.14	35.28 ± 2.95	38.01 ± 4.24	36.98 ± 4.76	36.74 ± 6.93
Total n-3	9.92 ± 1.77	9.65 ± 1.36	10.61 ± 2.17	10.19 ± 2.42	10.00 ± 3.02
TotalFAs (mg/g)	20.26 ± 3.17	19.67 ± 3.94	26.30 ± 3.65	24.14 ± 8.51	19.96 ± 4.32

Data are expressed as mean ± SD (n=5).
**p<0.01 and ***p<0.01, between the CTL and WC rats in the corresponding group.

Table 3.12 Fatty Acid Composition of liver PL of rats from MF group

Fatty Acids	CTL		
	Day 0	Day 17	Day 34
14:0	0.13 ± 0.03	0.13 ± 0.03	0.17 ± 0.05
16:0	14.05 ± 0.61	11.57 ± 2.54	13.09 ± 3.55
16:1n-7	0.51 ± 0.23	0.44 ± 0.09	0.43 ± 0.26
18:0	21.50 ± 1.76	21.23 ± 1.63	21.84 ± 2.57
18:1n-9	8.84 ± 1.17	8.40 ± 0.63	8.32 ± 2.09
18:1n-7	5.11 ± 0.61	4.91 ± 0.40	3.86 ± 0.33
18:2n-6	12.02 ± 0.98	9.65 ± 0.79	8.88 ± 0.22
20:3n-6	1.27 ± 0.25	0.97 ± 0.09	0.80 ± 0.28
20:4n-6	22.59 ± 2.49	25.42 ± 2.17	27.65 ± 2.86
22:4n-6	0.27 ± 0.03	0.19 ± 0.02	0.20 ± 0.04
22:5n-6	0.34 ± 0.03	0.24 ± 0.09	0.16 ± 0.04
18:3n-3	0.34 ± 0.07	0.33 ± 0.05	0.38 ± 0.03
20:5n-3	1.33 ± 0.10	1.12 ± 0.37	1.14 ± 0.30
22:5n-3	0.99 ± 0.20	1.00 ± 0.20	1.12 ± 0.08
22:6n-3	7.14 ± 1.12	7.49 ± 0.86	7.14 ± 1.24
Total SFAs	36.70 ± 1.93	33.99 ± 4.00	36.42 ± 2.08
Total MUFAs	15.78 ± 0.78	15.07 ± 1.07	13.63 ± 2.26
Total n-6	36.50 ± 3.14	36.47 ± 2.74	37.69 ± 3.15
Total n-3	9.80 ± 1.00	9.95 ± 1.37	9.78 ± 1.61
Total FAs (mg/g)	21.96 ± 1.15	24.96 ± 7.11	21.91 ± 0.53

Data are expressed as mean ± SD (n=5).

continued

Fatty Acids	WC 1				
	Day 1	Day 7	Day 8	Day 12	Day 17
14:0	0.13 ± 0.06	0.12 ± 0.04	0.12 ± 0.08	0.15 ± 0.03	0.15 ± 0.03
16:0	15.54 ± 4.19	15.00 ± 0.85	15.00 ± 5.10	12.73 ± 3.00	12.18 ± 1.23
16:1n-7	0.46 ± 0.23	0.39 ± 0.11	0.42 ± 0.36	0.49 ± 0.11	0.44 ± 0.04
18:0	22.32 ± 1.98	21.73 ± 1.82	21.58 ± 1.02	21.13 ± 3.31	22.12 ± 1.29
18:1n-9	7.65 ± 1.90	7.02 ± 0.97	6.71 ± 0.41	6.45 ± 0.42	7.58 ± 0.81
18:1n-7	4.70 ± 0.87	3.94 ± 0.32**	4.15 ± 0.23	4.76 ± 0.35	4.72 ± 1.20
18:2n-6	10.61 ± 0.98	11.41 ± 1.38	12.60 ± 0.82	10.46 ± 1.15	9.72 ± 0.69
20:3n-6	1.25 ± 0.17	1.17 ± 0.33	1.10 ± 0.14	1.03 ± 0.20	1.04 ± 0.05
20:4n-6	23.99 ± 4.41	22.59 ± 2.10	22.49 ± 2.53	24.30 ± 2.60	28.73 ± 0.93
22:4n-6	0.26 ± 0.02	0.17 ± 0.02***	0.28 ± 0.02	0.25 ± 0.03	0.21 ± 0.02
22:5n-6	0.37 ± 0.23	0.38 ± 0.05	0.30 ± 0.04	0.28 ± 0.13	0.24 ± 0.02
18:3n-3	0.34 ± 0.06	0.40 ± 0.14	0.42 ± 0.05	0.44 ± 0.13	0.31 ± 0.01
20:5n-3	1.04 ± 0.37	1.07 ± 0.32	1.14 ± 0.27	1.12 ± 0.35	1.10 ± 0.05
22:5n-3	1.02 ± 0.13	0.90 ± 0.12	0.95 ± 0.16	0.99 ± 0.07	0.95 ± 0.05
22:6n-3	6.33 ± 1.68	6.59 ± 0.68	5.76 ± 1.32	7.25 ± 1.72	7.43 ± 0.57
Total SFAs	39.02 ± 2.45	37.88 ± 1.64	37.70 ± 5.27	34.99 ± 1.36	35.32 ± 2.32
Total MUFAs	14.13 ± 1.05	12.59 ± 1.20**	12.31 ± 0.59	12.73 ± 0.48	13.49 ± 0.37*
Total n-6	36.47 ± 4.23	35.73 ± 1.08	36.77 ± 2.80	36.04 ± 1.60	39.94 ± 0.69*
Total n-3	8.73 ± 1.49	8.95 ± 0.53	8.27 ± 1.37	10.07 ± 2.02	9.79 ± 0.61
Total FAs (mg/g)	23.22 ± 2.38	17.97 ± 2.66*	19.02 ± 1.59	21.43 ± 0.80	23.44 ± 0.78

Data are expressed as mean ± SD (n=5).
*p<0.05, **p<0.01 and ***p<0.001, between the CTL and WC rats in the corresponding group.

continued

Fatty Acids	WC 2				
	Day 18	**Day 24**	**Day 25**	**Day 29**	**Day 34**
14:0	0.13 ± 0.08	0.12 ± 0.07	0.12 ± 0.07	0.15 ± 0.04	0.17 ± 0.05
16:0	11.55 ± 3.52	11.94 ± 1.70	12.15 ± 1.82	12.41 ± 2.27	12.89 ± 4.63
16:1n-7	0.48 ± 0.18	0.45 ± 0.12	0.46 ± 0.21	0.46 ± 0.18	0.41 ± 0.24
18:0	20.86 ± 1.26	22.53 ± 0.83	23.32 ± 1.35	22.18 ± 2.43	22.60 ± 6.46
18:1n-9	6.72 ± 1.89	6.51 ± 0.58	7.41 ± 1.50	7.64 ± 0.60	8.46 ± 1.24
18:1n-7	4.84 ± 1.10	4.36 ± 0.36	4.05 ± 0.18	3.96 ± 0.35	3.75 ± 1.25
18:2n-6	9.85 ± 1.02	9.87 ± 0.35	8.29 ± 0.62	9.77 ± 2.02	9.01 ± 2.33
20:3n-6	1.11 ± 0.25	1.16 ± 0.30	1.16 ± 0.11	1.03 ± 0.20	1.24 ± 0.31*
20:4n-6	22.36 ± 4.02	24.06 ± 1.58	25.26 ± 1.30	25.42 ± 2.01	24.48 ± 5.68
22:4n-6	0.21 ± 0.05	0.32 ± 0.02	0.32 ± 0.04	0.20 ± 0.05	0.22 ± 0.05
22:5n-6	0.27 ± 0.04	0.28 ± 0.14	0.25 ± 0.01	0.27 ± 0.08	0.27 ± 0.09
18:3n-3	0.41 ± 0.04	0.39 ± 0.07	0.34 ± 0.06	0.29 ± 0.07	0.37 ± 0.13
20:5n-3	1.13 ± 0.43	1.18 ± 0.59	1.15 ± 0.28	1.13 ± 0.35	1.20 ± 0.46
22:5n-3	1.28 ± 0.08	1.04 ± 0.13	1.13 ± 0.27	1.12 ± 0.21	1.16 ± 0.25
22:6n-3	6.46 ± 0.61	7.72 ± 1.20	7.74 ± 1.12	7.47 ± 1.34	6.91 ± 2.04
Total SFAs	33.32 ± 3.79	35.29 ± 1.01	36.39 ± 1.77	35.62 ± 1.13	36.64 ± 10.03
Total MUFAs	13.04 ± 0.91	12.03 ± 0.68	12.91 ± 1.58	13.04 ± 0.98	13.34 ± 1.03
Total n-6	33.80 ± 4.77	35.69 ± 1.37	35.27 ± 1.46	36.69 ± 1.75	35.21 ± 7.71
Total n-3	9.27 ± 0.54	10.34 ± 1.64	10.36 ± 1.14	10.01 ± 1.13	9.64 ± 2.45
Total FAs (mg/g)	18.55 ± 2.43	21.40 ± 6.73	21.56 ± 2.07	23.98 ± 8.35	22.19 ± 3.59

Data are expressed as mean ± SD (n=5).
*$p<0.05$, between the CTL and WC rats in the corresponding group.

3.4.7.3.3 Liver TG

At the end of two WCs, there was no marked difference in the fatty acid composition of liver TG between the WC and CTL rats fed HF diet (Table 3.13). Nevertheless, during food restriction, 18:2n-6 and 18:3n-3 were essentially increased in WC rats. In contrast, 18:1n-9 was greatly reduced in the WC rats during the second food restriction.

In MF group, no difference was found in the liver TG fatty acid composition between WC and CTL rats after weight cycling (Table 3.14). However, during food restriction, the fatty acid profiles were substantially remodelled in the WC rats. Significant decrease of 16:0, 16:1n-7, 18:1n-7 and 22:6n-3 occurred in WC rats. Furthermore, a marked increase of 18:2n-6 and 18:3n-3 was also found in WC rats during food restriction.

Table 3.13 Fatty Acid Composition of liver TG of rats from HF group

Fatty Acids	CTL				
	Day 0	**Day 7**	**Day 17**	**Day 24**	**Day 34**
14:0	1.18 ± 0.30	1.21 ± 0.20	1.08 ± 0.17	1.17 ± 0.22	1.19 ± 0.22
16:0	30.28 ± 1.06	28.38 ± 3.53	28.92 ± 3.61	25.22 ± 2.93	27.33 ± 2.89
16:1n-7	6.92 ± 2.03	6.20 ± 1.19	5.59 ± 0.97	5.75 ± 0.95	4.84 ± 1.20
18:0	2.13 ± 0.72	2.30 ± 0.96	2.34 ± 0.35	2.21 ± 0.52	2.56 ± 0.91
18:1n-9	32.01 ± 3.88	33.55 ± 2.58	37.79 ± 4.96	40.51 ± 4.28	44.31 ± 5.61
18:1n-7	6.39 ± 1.42	6.50 ± 1.23	5.97 ± 1.15	5.52 ± 0.84	4.89 ± 0.69
18:2n-6	9.38 ± 2.62	9.83 ± 2.35	8.96 ± 2.20	9.39 ± 1.42	8.11 ± 2.69
20:3n-6	0.36 ± 0.06	0.32 ± 0.04	0.27 ± 0.04	0.24 ± 0.04	0.18 ± 0.03
20:4n-6	0.20 ± 0.10	0.22 ± 0.04	0.28 ± 0.09	0.34 ± 0.07	0.40 ± 0.16
22:4n-6	0.16 ± 0.05	0.13 ± 0.04	0.16 ± 0.05	0.15 ± 0.07	0.15 ± 0.05
22:5n-6	0.06 ± 0.02	0.07 ± 0.02	0.07 ± 0.01	0.07 ± 0.04	0.05 ± 0.02
18:3n-3	0.86 ± 0.22	0.97 ± 0.17	1.12 ± 0.14	1.47 ± 0.31	1.47 ± 0.38
20:5n-3	0.23 ± 0.03	0.17 ± 0.04	0.13 ± 0.04	0.11 ± 0.02	0.10 ± 0.02
22:5n-3	0.35 ± 0.12	0.37 ± 0.11	0.35 ± 0.06	0.27 ± 0.07	0.21 ± 0.07
22:6n-3	0.75 ± 0.16	0.63 ± 0.14	0.54 ± 0.29	0.46 ± 0.12	0.37 ± 0.07
Total SFAs	34.21 ± 1.30	34.57 ± 3.92	33.58 ± 2.52	29.25 ± 2.64	31.74 ± 3.25
Total MUFAs	46.04 ± 6.28	47.81 ± 2.11	50.34 ± 6.18	52.77 ± 4.12	54.81 ± 5.31
Total n-6	10.16 ± 2.74	10.56 ± 2.41	9.74 ± 2.22	10.83 ± 1.38	8.88 ± 2.79
Total n-3	2.18 ± 0.32	2.14 ± 0.34	2.14 ± 0.29	2.30 ± 0.17	2.14 ± 0.43
Total FAs (mg/g)	13.40 ± 1.36	13.76 ± 0.29	14.10 ± 0.12	14.24 ± 0.28	14.24 ± 0.28

Data are expressed as mean ± SD (n=5).

continued

Fatty Acids	WC 1				
	Day 1	**Day 7**	**Day 8**	**Day 12**	**Day 17**
14:0	1.11 ± 0.21	1.10 ± 0.38	1.05 ± 0.32	1.15 ± 0.21	1.14 ± 0.37
16:0	29.76 ± 5.14	27.05 ± 5.65	27.66 ± 2.60	27.28 ± 2.86	28.99 ± 3.14
16:1n-7	6.48 ± 1.15	4.70 ± 0.41	5.02 ± 0.52	5.13 ± 1.00	5.99 ± 1.85
18:0	2.27 ± 0.39	2.14 ± 0.37	1.84 ± 0.29	2.30 ± 0.18	2.52 ± 0.56
18:1n-9	32.41 ± 6.26	30.51 ± 4.25	32.81 ± 6.68	33.71 ± 2.61	35.67 ± 4.20
18:1n-7	6.33 ± 2.48	5.90 ± 0.72	5.26 ± 0.42	5.74 ± 0.25	5.91 ± 1.11
18:2n-6	9.73 ± 3.12	14.08 ± 2.42*	11.23 ± 1.37	9.21 ± 1.80	9.27 ± 1.81
20:3n-6	0.32 ± 0.13	0.31 ± 0.10	0.25 ± 0.07	0.21 ± 0.02	0.23 ± 0.04
20:4n-6	0.25 ± 0.09	0.24 ± 0.06	0.25 ± 0.07	0.25 ± 0.11	0.32 ± 0.06
22:4n-6	0.16 ± 0.05	0.15 ± 0.04	0.13 ± 0.05	0.14 ± 0.06	0.16 ± 0.06
22:5n-6	0.07 ± 0.02	0.06 ± 0.03	0.05 ± 0.02	0.06 ± 0.03	0.07 ± 0.02
18:3n-3	0.96 ± 0.34	1.41 ± 0.24*	1.44 ± 0.43	1.29 ± 0.38	1.14 ± 0.10
20:5n-3	0.24 ± 0.07	0.17 ± 0.05	0.17 ± 0.05	0.15 ± 0.03	0.12 ± 0.02
22:5n-3	0.33 ± 0.06	0.32 ± 0.06	0.31 ± 0.06	0.25 ± 0.02	0.28 ± 0.06
22:6n-3	0.57 ± 0.11	0.55 ± 0.12	0.52 ± 0.08	0.52 ± 0.11	0.53 ± 0.15
Total SFAs	33.66 ± 5.41	31.12 ± 5.21	31.24 ± 2.73	31.36 ± 3.01	33.30 ± 2.53
Total MUFAs	45.98 ± 4.49	42.11 ± 4.93	43.68 ± 6.64	45.57 ± 2.20	48.85 ± 5.55
Total n-6	10.52 ± 3.26	14.85 ± 2.37*	11.92 ± 1.36	9.86 ± 1.85	10.03 ± 1.78
Total n-3	2.10 ± 0.50	2.44 ± 0.13	2.44 ± 0.38	2.21 ± 0.44	2.07 ± 0.21
Total FAs (mg/g)	13.59 ± 1.08	13.96 ± 0.83	14.43 ± 0.14	13.96 ± 0.27	14.38 ± 0.13

Data are expressed as mean ± SD (n=5).
*$p<0.05$, between the CTL and WC rats in the corresponding group.

continued

Fatty Acids	WC 2				
	Day 18	Day 24	Day 25	Day 29	Day 34
14:0	1.29 ± 0.11	1.06 ± 0.31	1.21 ± 0.46	1.33 ± 0.40	1.23 ± 0.31
16:0	24.82 ± 3.02	25.54 ± 4.95	30.66 ± 1.54	29.30 ± 5.96	28.54 ± 4.38
16:1n-7	5.06 ± 0.77	4.48 ± 0.72	5.73 ± 2.64	5.76 ± 1.57	5.31 ± 1.32
18:0	2.07 ± 0.54	1.94 ± 0.24	2.13 ± 0.58	2.08 ± 0.28	2.49 ± 0.42
18:1n-9	35.02 ± 4.21	27.53 ± 5.60**	31.16 ± 4.85	39.14 ± 8.65	42.35 ± 4.30
18:1n-7	5.74 ± 1.42	5.00 ± 0.73	5.31 ± 0.44	5.63 ± 1.08	5.16 ± 0.76
18:2n-6	9.88 ± 2.18	14.05 ± 2.13**	10.59 ± 3.65	8.97 ± 2.78	8.09 ± 1.70
20:3n-6	0.21 ± 0.04	0.19 ± 0.04	0.17 ± 0.04	0.18 ± 0.03	0.17 ± 0.03
20:4n-6	0.29 ± 0.04	0.30 ± 0.07	0.39 ± 0.08	0.42 ± 0.21	0.37 ± 0.14
22:4n-6	0.16 ± 0.05	0.14 ± 0.06*	0.15 ± 0.02	0.12 ± 0.05	0.14 ± 0.04
22:5n-6	0.05 ± 0.02	0.05 ± 0.02	0.07 ± 0.01	0.06 ± 0.03	0.07 ± 0.03
18:3n-3	1.28 ± 0.15	2.08 ± 0.30	1.77 ± 0.40	1.55 ± 0.46	1.37 ± 0.24
20:5n-3	0.14 ± 0.03	0.10 ± 0.04	0.13 ± 0.06	0.13 ± 0.07	0.13 ± 0.06
22:5n-3	0.20 ± 0.02	0.23 ± 0.08	0.18 ± 0.11	0.16 ± 0.09	0.13 ± 0.03
22:6n-3	0.41 ± 0.11	0.39 ± 0.09	0.34 ± 0.11	0.31 ± 0.11	0.30 ± 0.06
Total SFAs	28.83 ± 3.11	29.32 ± 4.72	34.87 ± 2.30	33.27 ± 6.05	32.91 ± 4.74
Total MUFAs	46.39 ± 4.10	37.74 ± 6.50**	43.03 ± 6.63	51.87 ± 9.19	54.09 ± 3.22
Total n-6	10.59 ± 2.25	14.73 ± 2.11**	11.38 ± 3.69	9.75 ± 2.92	8.83 ± 1.84
Total n-3	2.04 ± 0.20	2.80 ± 0.39	2.43 ± 0.41	2.15 ± 0.46	1.92 ± 0.25
Total FAs (mg/g)	13.70 ± 0.80	13.67 ± 0.55	14.75 ± 0.36	13.84 ± 0.96	14.18 ± 0.07

Data are expressed as mean ± SD (n=5).
*$p<0.05$ and **$p<0.01$, between the CTL and WC rats in the corresponding group.

Table 3.14 Fatty Acid Composition of liver TG of rats from MF group

Fatty Acids	CTL		
	Day 0	**Day 17**	**Day 34**
14:0	1.14 ± 0.16	0.92 ± 0.19	0.93 ± 0.29
16:0	34.48 ± 4.15	27.81 ± 2.26	26.03 ± 2.75
16:1n-7	6.81 ± 2.13	3.89 ± 1.25	3.66 ± 1.67
18:0	2.03 ± 0.41	2.25 ± 0.34	2.44 ± 0.87
18:1n-9	34.98 ± 1.59	40.12 ± 1.55	44.29 ± 3.08
18:1n-7	6.17 ± 0.67	4.56 ± 0.37	4.68 ± 0.31
18:2n-6	8.90 ± 3.76	8.62 ± 2.11	7.72 ± 2.56
20:3n-6	0.35 ± 0.06	0.20 ± 0.08	0.11 ± 0.06
20:4n-6	0.19 ± 0.07	0.27 ± 0.09	0.38 ± 0.19
22:4n-6	0.15 ± 0.05	0.15 ± 0.05	0.10 ± 0.06
22:5n-6	0.07 ± 0.02	0.07 ± 0.01	0.07 ± 0.02
18:3n-3	0.79 ± 0.44	1.25 ± 0.27	1.40 ± 0.36
20:5n-3	0.22 ± 0.03	0.13 ± 0.09	0.09 ± 0.06
22:5n-3	0.34 ± 0.13	0.24 ± 0.06	0.16 ± 0.06
22:6n-3	0.73 ± 0.12	0.42 ± 0.28	0.26 ± 0.03
Total SFAs	38.27 ± 4.01	32.80 ± 1.29	30.06 ± 3.14
Total MUFAs	48.67 ± 3.40	49.52 ± 2.83	53.40 ± 3.00
Total n-6	9.65 ± 3.73	9.31 ± ± 2.12	8.39 ± 2.70
Total n-3	2.09 ± 0.39	2.04 ± 0.30	1.91 ± 0.43
Total FAs (mg/g)	13.93 ± 0.45	13.22 ± 0.11	13.24 ± 0.26

Data are expressed as mean ± SD (n=5).

continued

Fatty Acids	WC 1				
	Day 1	Day 7	Day 8	Day 12	Day 17
14:0	1.07 ± 0.24	0.95 ± 0.33	0.92 ± 0.47	1.27 ± 0.20	1.09 ± 0.50
16:0	31.04 ± 3.86	16.56 ± 1.76***	26.86 ± 0.62	24.54 ± 2.77	27.59 ± 0.35
16:1n-7	6.28 ± 1.35	1.48 ± 0.36***	4.87 ± 0.51	4.78 ± 0.97	3.70 ± 0.05
18:0	1.70 0.19	2.14 ± 0.21	1.49 ± 0.08	1.74 ± 0.18	2.40 ± 0.53
18:1n-9	32.66 ± 14.78	37.88 ± 3.66	41.54 ± 1.70	42.44 ± 2.54	42.54 ± 0.55*
18:1n-7	6.69 ± 3.04	5.13 ± 0.33*	5.11 ± 0.41	5.57 ± 0.24	4.25 ± 0.09
18:2n-6	8.36 ± 4.18	14.81 ± 0.74**	10.91 ± 1.33	8.94 ± 1.75	8.83 ± 0.43
20:3n-6	0.30 ± 0.10	0.27 ± 0.17	0.25 ± 0.15	0.20 ± 0.02	0.19 ± 0.02
20:4n-6	0.24 ± 0.09	0.21 ± 0.05	0.24 ± 0.07	0.24 ± 0.11	0.30 ± 0.01
22:4n-6	0.15 ± 0.06	0.09 ± 0.07	0.11 ± 0.07	0.13 ± 0.06	0.15 ± 0.05
22:5n-6	0.06 ± 0.02	0.06 ± 0.02	0.07 ± 0.02	0.05 ± 0.03	0.05 ± 0.03
18:3n-3	0.92 ± 0.33	1.92 ± 0.59**	2.41 ± 0.21	1.45 ± 0.33	1.05 ± 0.01
20:5n-3	0.22 ± 0.11	0.14 ± 0.05	0.14 ± 0.08	0.13 ± 0.02	0.11 ± 0.02
22:5n-3	0.31 ± 0.10	0.28 ± 0.05	0.25 ± 0.05	0.24 ± 0.02	0.22 ± 0.02
22:6n-3	0.47 ± 0.13	0.35 ± 0.08**	0.41 ± 0.02	0.37 ± 0.04	0.31 ± 0.03
Total SFAs	34.34 ± 4.04	20.49 ± 1.32***	29.96 ± 0.95	28.18 ± 3.02	31.73 ± 0.52
Total MUFAs	46.40 ± 12.47	45.48 ± 4.47	52.11 ± 2.12	53.78 ± 2.14	51.77 ± 0.55
Total n-6	9.12 ± 4.30	15.45 ± 0.73**	11.57 ± 1.27	9.57 ± 1.79	9.51 ± 0.41
Total n-3	1.92 ± 0.66	2.68 ± 0.52	3.21 ± 0.27	2.19 ± 0.37	1.70 ± 0.05
Total FAs (mg/g)	12.96 ± 2.33	11.87 ± 0.71**	13.67 ± 0.13	13.23 ± 0.25	13.37 ± 0.12

Data are expressed as mean ± SD (n=5).
*p<0.05, **p<0.01 and ***p<0.001, between the CTL and WC rats in the corresponding group.

continued

Fatty Acids	WC 2				
	Day 18	Day 24	Day 25	Day 29	Day 34
14:0	1.25 ± 0.11	1.05 ± 0.41	1.59 ± 0.42	1.53 ± 0.35	1.06 ± 0.30
16:0	24.10 ± 0.24	24.31 ± 3.15	30.87 ± 2.33	27.61 ± 5.43	28.14 ± 4.17
16:1n-7	2.97 ± 0.28	2.24 ± 0.66	5.31 ± 2.44	5.44 ± 1.51	4.65 ± 1.36
18:0	2.01 ± 0.53	1.77 ± 0.22	1.79 ± 0.54	1.97 ± 0.30	2.79 ± 0.19
18:1n-9	43.70 ± 4.09	37.21 ± 2.95	36.37 ± 4.44	37.07 ± 8.69	40.34 ± 4.10
18:1n-7	4.60 ± 0.09	3.89 ± 0.19	4.91 ± 0.41	4.37 ± 0.47	4.91 ± 0.13
18:2n-6	9.59 ± 2.12	11.28 ± 1.53	9.81 ± 3.38	8.17 ± 2.91	7.58 ± 1.76
20:3n-6	0.18 ± 0.01	0.15 ± 0.06	0.16 ± 0.04	0.12 ± 0.02	0.11 ± 0.02
20:4n-6	0.28 ± 0.02	0.22 ± 0.02	0.27 ± 0.08	0.40 ± 0.20	0.35 ± 0.13
22:4n-6	0.15 ± 0.05	0.14 ± 0.07	0.11 ± 0.01	0.11 ± 0.05	0.10 ± 0.05
22:5n-6	0.05 ± 0.02	0.05 ± 0.02	0.06 ± 0.01	0.05 ± 0.03	0.06 ± 0.03
18:3n-3	1.24 ± 0.15	1.73 ± 0.38	1.01 ± 0.61	1.23 ± 0.64	1.02 ± 0.14
20:5n-3	0.09 ± 0.01	0.14 ± 0.09	0.15 ± 0.09	0.12 ± 0.06	0.12 ± 0.06
22:5n-3	0.20 ± 0.02	0.21 ± 0.12	0.17 ± 0.10	0.15 ± 0.09	0.12 ± 0.01
22:6n-3	0.30 ± 0.02	0.15 ± 0.10	0.22 ± 0.18	0.20 ± 0.02	0.17 ± 0.02***
Total SFAs	28.01 ± 0.81	27.91 ± 2.92	35.11 ± 2.86	31.68 ± 5.49	32.63 ± 4.37
Total MUFAs	51.85 ± 4.08	44.05 ± 2.96	47.43 ± 6.17	48.21 ± 8.82	51.17 ± 2.83
Total n-6	10.26 ± 2.16	11.84 ± 1.48	10.41 ± 3.42	8.86 ± 3.03	8.20 ± 1.90
Total n-3	1.83 ± 0.18	2.22 ± 0.34	1.54 ± 0.57	1.70 ± 0.70	1.43 ± 0.17
Total FAs (mg/g)	12.98 ± 0.76	12.14 ± 0.49	13.34 ± 0.32	12.77 ± 1.07	13.19 ± 0.06

Data are expressed as mean ± SD (n=5).

***$p<0.001$, between the CTL and WC rats in the corresponding group.

3.5 Discussion

3.5.1 Weight Cycling-Induced Obesity Only with a High-Fat Diet

The present results suggested that weight cycling could cause an increase in weight of adipose tissue when the rats were fed a constant high-fat diet (45% of total calories) (Figures 3.6 and 3.7). This was because the size of adipocytes was considerably enlarged (80% increase) in WC rats fed HF diet when compared with the CTL rats (Figure 3.16), though the number of adipocytes remained unchanged. This was in contrast to the previous study (Chapter 2), together with other reports, in which weight cycling was carried out with a constant low-fat diet (Hill *et al.,* 1987; 1988; Wheelers *et al.,* 1990), and an increase in the size of adipose tissues was not observed. Weight cycling with a constant medium-fat diet (22% of total calories) in this study did not increase any adiposity as well. Therefore, the results emphasized that "weight cycling - induced obesity" seemed to be true only when rats were fed a constant high-fat diet.

This finding may be explained by the fact that dietary fat is less oxidizable and has high caloric value compared with carbohydrate (Verboeket-van deVenne *et al.,* 1994). This explanation has been further supported recently by Horton *et al.* (1995) who showed that a high-fat diet did not necessarily increase oxidation of fat as compared with carbohydrates. It appeared that the excess fat were stored and did not produce significant alteration in the overall pattern of substrate utilization. In contrast, overfeeding with a lower fat but higher carbohydrate diet rapidly disrupted the existing pattern of substrate utilization, in which carbohydrate oxidation and total energy expenditure were increased.

It is also well documented that dietary fat can lead to adiposity due to the energy unbalance (Schutz *et al.,* 1992; Anonymous, 1988). Although most of the excess energy would be stored regardless of the source, it is important to note that more fat would be stored with high-fat diet than with an isocaloric carbohydrate diet during overfeeding. It has already been mentioned in Section 3.1.2 that dietary fat could produce a dose-response relationship with adiposity in rats fed isocalorically (Boozer *et al.,* 1995). In another study performed by Suzuki and co-workers (1990), the abdominal adipose weight and total body fat accumulation were found to be greater in rats with a high-fat dietary history (40% of total calories from fat) than in those with a low fat dietary history (5% of total energy from fat). Therefore, weight cycling with a high-fat diet is more potent in inducing obesity than weight cycling with either low-fat or medium-fat diets.

During the first few days of refeeding, the food intake by WC rats was increased. The food efficiency in the WC rats during refeeding was also significantly increased (Table 3.2). Therefore, the WC rats captured energy more efficiently, especially for the WC rats fed with a high-fat diet. For these rats, the considerable excessive dietary fat intake would be stored in the body and resulted in heavier fat pads than controls. WC rats fed a low or a medium-high fat diet also showed an increase in food intake and food efficiency during refeeding. However, the excessive energy intake was mainly from carbohydrate which was easily oxidized. Therefore, their replenished fat stores did not give a higher fat pad weight than that of their controls.

3.5.1.2 Effect of Weight Cycling on the Size of Adipocytes

The weight of adipose tissue is determined by both the number and size of the fat cells. However, the significantly higher weight of epididymal and perirenal fat pads in the WC rats fed a high-fat diet was only accounted by the enlarged size of adipocytes as the number of adipocytes remained unchanged. During refeeding after energy restriction, the fat cells were able to rebuild their energy stores and thus their size increased. The enlargement of adipocytes may firstly be due to the signals derived from adipocytes to lead rats to ingest more calories than they expend (Miller *et al.*, 1983). Secondly, as demonstrated by Fried *et al.* (1983a), the activity of adipose tissue lipoprotein lipase, which regulates the lipid uptake of adipocytes, can be considerably increased during refeeding after caloric restriction. Therefore, the excessive fat intake would then be efficiently stored up by the adipocytes. Thirdly, the *de novo* lipogenesis may also be elevated (Leveille and Hanson, 1965). Moreover, the enlarged metabolically active surface of the fat cells may further increase the cell size since the metabolic activity is best correlated to the cell surface (Bjorntorp and Sjostrom, 1972). The possible changes in hormone receptors, such as insulin receptors, on the cell surface may also subsequently cause the enlargement of the cells (Olefsky, 1976).

The present study showed that weight cycling, no matter with a high-fat or a medium-fat diet, did not give an increase in the number of adipocytes. This seems to be inconsistent with previous studies which demonstrated that feeding a high-fat diet (50% energy) after fasting could promote the formation of new fat cells in adipose tissue (Ailhaud *et al.*, 1992). This discrepancy may be explained by the fact that a certain critical fat cell size, which indicates the adipocytes are "full", must first be

reached before new fat cells can be formed (Lemonnier, 1972). It has been shown that fat-feeding could increase the turnover rate of adipoblasts. These adipoblasts were prevented from being committed to and included in the preadipocyte-adipocyte pool of cells to a certain limit where the inhibition on the factors determining development was released and preadipocytes could be formed. They were then rapidly filled with lipid and included in the adipocyte pool (Bjorntorp and Sjostrom, 1972). Thus, it is possible that the fat cell size of the WC rats in this study has not attained such a critical value yet, and so, no change in adipocyte number could be observed. Furthermore, the Sprague-Dawley rats we used as the experimental model was known to be less sensitive to fat-feeding and proliferation of the adipocytes was less pronounced (Lemonier, 1972). Therefore, the size but not the number of adipocytes in the WC rats fed high-fat diet was increased in the present study. Moreover, some studies argued that fasting followed by refeeding typically has little effect on the number of adipocytes (Bernloh and Simpson, 1996; Miller *et al.,* 1983).

3.5.1.3 Food Efficiency during Weight Cycling

As shown in Table 3.2, the food efficiency of WC rats in cycle 1 and cycle 2 was similar. Their food efficiency in the first refeeding was also close to that in the second refeeding. Therefore, the present results did not demonstrate that the second WC would increase food efficiency compared with the first WC. However, the food efficiency in the two refeeding periods was significantly higher in WC rats than that of the controls (Table 3.2).

3.5.2 Weight-Cycling Induced Specific Alteration of Fatty Acid Metabolism

The present results clearly demonstrated that two WCs remodelled the fatty acid composition of adipose tissue and carcass lipids (Figures 3.23, 3.24, 3.25, 3.26, 3.31, 3.32, 3.33 and 3.34). This change was also specific and regardless of the level of dietary fat. 18:2n-6 and 18:3n-3 were proportionally depleted, whereas the content of saturated and monounsaturated fatty acids, including 14:0, 16:0, 16:1n-7 and 18:0, were concomitantly increased in the adipose tissue and carcass in WC rats fed with either a high-fat (45% of total calories from fat) or medium-fat (22% of total calories from fat) diet. This alteration markedly lowered the ratio of polyunsaturated to saturated fatty acids. This may enhance the incidence of CHD if humans response similarly to weight cycling as rats. Moreover, this remodelling was consistent with previous studies which showed that specific reduction of 18:2n-6 and 18:3n-3 induced by weight cycling was also independent ofthe extent of food restriction, animal age and dietary levels of linoleic acid (Chen *et al.,* 1995; 1996; 1997; Chen and Ratnayake, 1995; Chen and Cunnane, 1993). The alteration of the fatty acid profile of carcass total lipids was mainly due to the change in the fatty acid composition of carcass TG.

The specific alteration of the fatty acid profile in the adipose tissue and carcass total lipids may be explained by the mechanism proposed in Chapter 2. During fasting, 18:2n-6 and 18:3n-3 were preferentially mobilized from adipose tissue and carcass TG. They were then oxidized relatively faster than saturated and monounsaturated fatty acids. However, refeeding led to an increase in the synthesis of saturated and monounsaturated fatty acids. Thus, an episode of energy restriction

followed by refeeding would result in a proportional decrease in 18:2n-6 and 18:3n-3 and a proportional increase in saturated and monounsaturated fatty acids including mainly 14:0, 16:0, 16:1n-7 and 18:0.

It was found that alteration of the fatty acid composition occurred mainly in carcass TG. In contrast, no such observations were found in the carcass PL and carcass FFA. Therefore, the alteration of the fatty acid profile of carcass total lipids was due to the remodelling of the fatty acids composition of carcass TG.

The fatty acid composition of liver total lipids was shown to have no difference between WC and CTL rats at the end of two WCs and this was consistent with previous study (Chen *et al.,* 1997). In contrast, there was a remodelling in fatty acid profile of liver TG during the phase of energy restriction and this change was opposite to that in adipose tissue and carcass total lipids. On day 7 and day 24, which were the seventh day of the first and second energy restriction, respectively, the proportions of 18:2n-6 and 18:3n-3 were increased in the liver TG instead. This was also true in WC rats fed MF diet. The present observations were supported by previous studies (Cunnane, 1988; Chen and Cunnane, 1991; 1992). It has been suggested that during energy deficit, liver TG species were degraded differentially. The lower molecular weight and less unsaturated TG components (e.g. 14:0, 16:0, 16:1n-7) were depleted faster than the higher molecular weight and more unsaturated ones such as 18:2n-6 and 18:3n-3 - containing TG species. Thus, liver TG composed of or enriched with palmitic and palmitoleic acids served as an important liver lipid source for energy under fasting or starvation (Chen and Cunnane, 1991). As oxidation of exogenous free 18:2n-6 and 18:3n-3 during fasting was much slower (Bjorntorp,

1968), it has been proposed that reacylation of these fatty acids with the monolinoleoyl and monolinolenoyl mobilized from the adipose tissue occurred (Chen and Cunnane, 1992). As a result, 18:2n-6 and 18:3n-3 were preferentially retained in the liver TG.

The fatty acid composition of both carcass PL and liver PL remained almost unchanged throughout the study. It is believed that PL plays an important physiological role in membrane and thus, the fatty acids comprising the PL were comparatively inert to be mobilized and oxidized during energy restriction.

Chapter 4

Weight Cycling Altered the Activities of Lipoprotein Lipase and Lipogenic Enzymes in Rats

4.1 Introduction

4.1.1 Fatty Acid Metabolism

Fatty acids are physiologically important. First of all, fatty acids are fuel molecules and are stored as triacylglycerols. They are also the building blocks of phospholipids and glycolipids. Furthermore, the covalent attachment of fatty acids modifies many proteins for their allocations on membranes. Besides, some fatty acid derivatives serve as hormones and intracellular messengers as mentioned in Section 2.1.2, Chapter 2.

4.1.1.1 Fatty Acid Synthesis

Fatty acids are synthesized in the cytosol. Fatty acid biosynthesis occurs through condensation of C_2 units and acetyl-CoA was the precursor of the condensation reaction. The process involves two steps. The first step is the ATP-dependent carboxylation of acetyl-CoA to malonyl-CoA catalyzed by acetyl-CoA carboxylase (ACC). The sequence of reaction is shown in Figure 4.1. This is an irreversible reaction and is the committed step in fatty acid synthesis. The second step is the exergonic decarboxylation of the malonyl group in the condensation reaction (elongation) in the presence of acetyl-CoA and NADPH. This step is catalyzed by an enzyme system called fatty acid synthase (FAS). The reaction involves condensation

of acetyl and malonyl to form aceto-acetyl group with the release of CO_2 from the activated malonyl unit. This is followed by a reduction, a dehydration and a second reduction (Figure 4.1). A butyryl group is formed in this way and is ready for a second round of elongation, starting with the addition of a C_2 unit from malonyl-CoA. Seven rounds of elongation yield palmitoyl, which is then hydrolyzed to palmitate. The synthesis of palmitate requires 8 molecules of acetyl-CoA, 14 NADPH, and 7 ATP. Acetyl-CoA is formed from pyruvate in the mitochodria and is transported into the cytosol through the pyruvate/malate cycle (Figure 4.2). The pyruvate is produced from glucose via glycolysis. The formation of pyruvate from phosphoenolpyruvate is catalyzed by pyruvate kinase (PK), which is also a key enzyme that can regulate the provision of precursors for fatty acid synthesis. Besides the pentose phosphate shunt, the pyruvate/malate cycle also generates NADPH which is needed for fatty acid synthesis. As shown in Figure 4.3, malate is oxidatively decarboxylated by malic enzyme (ME) with the formation of NADPH and pyruvate thus completing the cycle. The fatty acids are then esterified and condensed with glycerol-3-phosphate to form triglycerides.

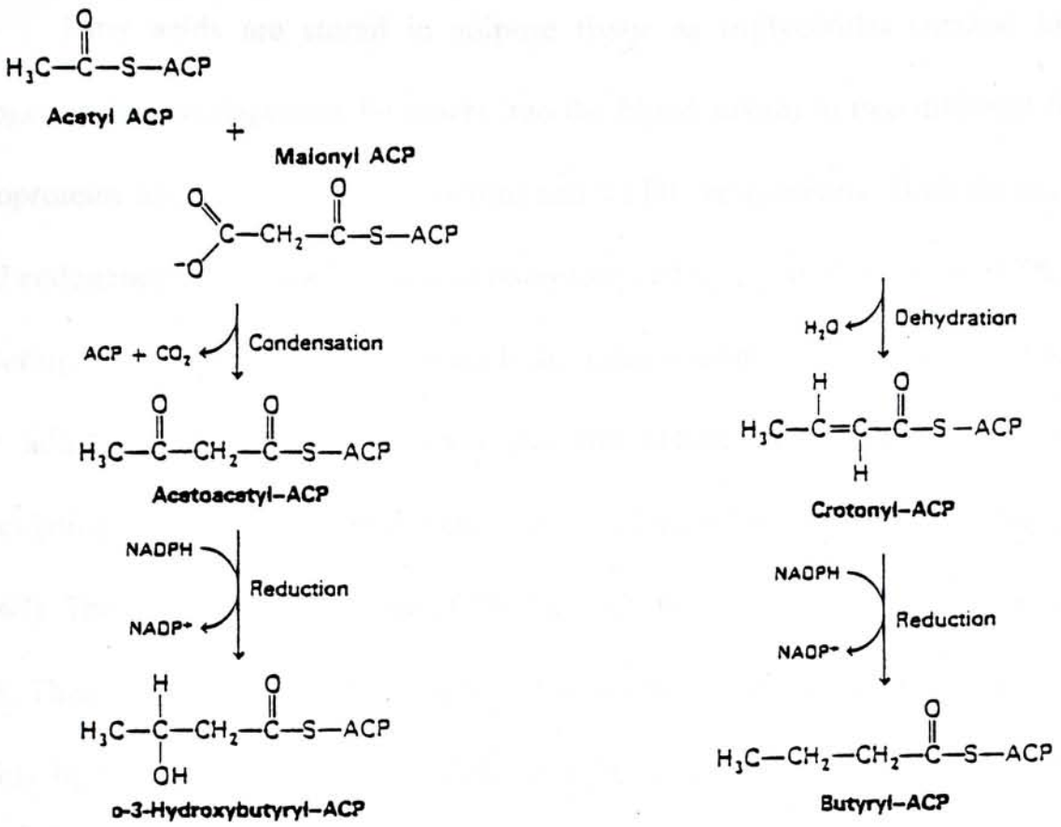


Figure 4.1 Reaction sequence of the fatty acid biosynthesis.

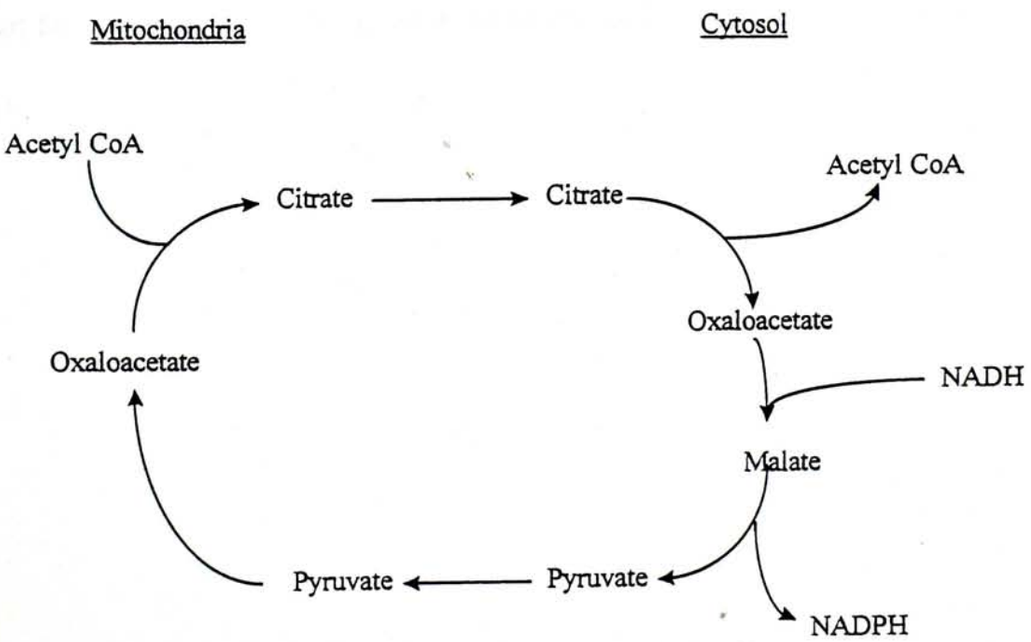


Figure 4.2 Pyruvate-malate cycle.

4.1.1.2 Fatty Acid Storage

Fatty acids are stored in adipose tissue as triglycerides (neutral fat). The exogenous and endogenous fat enters into the blood stream in two different forms of lipoproteins and are called chylomicrons and VLDL, respectively. Both the exogenous and endogenous triglycerides have to be hydrolyzed by lipoprotein lipase (LPL) before entering the adipocytes. The fatty acids are then re-esterified and deposited as TG in the adipocytes. It has been shown that the uptake of fatty acids derived from circulating triacylglycerols is directly correlated with LPL activity (Grarfinkel *et al.,* 1967). The transport and storage of the triglyceride fatty acids are illustrated in Figure 4.3. Thus, it is clear that LPL regulates the utilization of circulating triglyceride fatty acids by various tissues. In the case of adipose tissue, this hydrolysis is the rate-limiting step in the deposition of triglyceride fatty acids. On the other hand, LPL activity varies with nutritional and physiological states such as fasting, diabetes and genetic factors (Scow *et al.,* 1976; Eckel, 1989; Ong and Kern, 1989). Adipocytes are the major sites of accumulation of TG in mammals. The TG droplets coalesce to form a large fat globule which occupies most of the cell volume (Bjorntorp and Sjostrom, 1972).

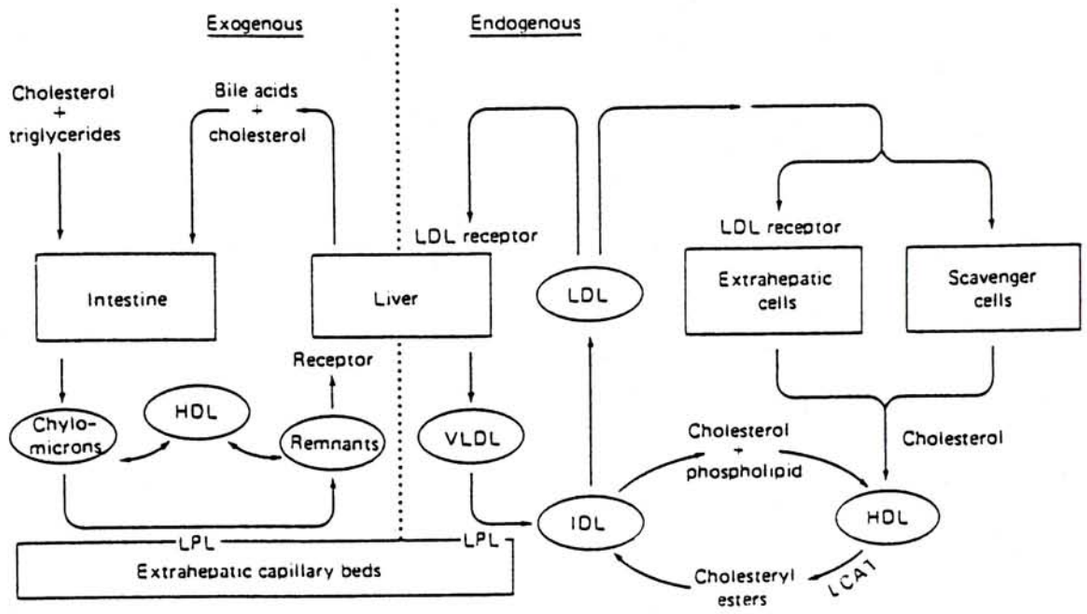


Figure 4.3 Transport and storage of triglyceride fatty acids (Linder, 1991).

4.1.1.3 Fatty Acid Oxidation

The initial event in the oxidation of stored triglyceride fatty acids is the hydrolysis of TG by hormone sensitive lipase (HSL). After that, fatty acids are mobilized and activated to acyl CoAs. The acyl CoAs are then transported across the inner mitochondrial membrane by carnitine, and are degraded in the mitochondrial matrix by the sequential removal of C_2 units. Each round of oxidation produces one NADH, one $FADH_2$ and one acetyl-CoA (Figure 4.4). Oxidation of acetyl-CoA via

the citric acid cycle generates additional $FADH_2$ and NADH, which are re-oxidized through oxidative phosphorylation to form ATP. Complete oxidation of a fatty acid molecule is therefore a highly exergonic process, and yields numerous ATPs.

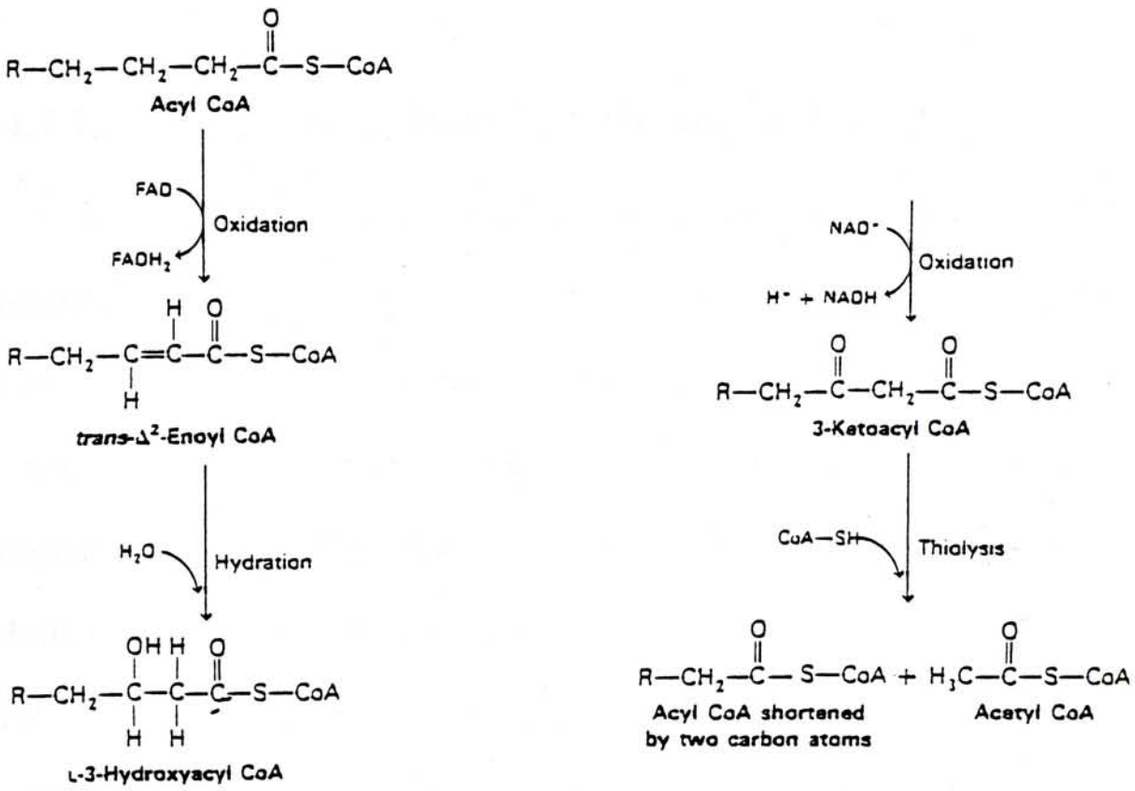


Figure 4.4 Reaction sequence of fatty acids degradation.

4.1.2 Hormonal Control of Fatty Acid Metabolism During Fasting and Refeeding

Fatty acid metabolism is stringently regulated. The synthesis and utilization of fatty acid are therefore highly responsive to physiologic needs. This is achieved by the actions of hormones.

4.1.2.1 Fatty Acid Metabolism During Fasting

During fasting, concentration of blood glucose falls which stimulates the pancreatic α-cells to release glucagon (Bender, 1995). Glucagon is the main hormone in the fasting state. It increases fatty acids mobilization from the triglycerides reserves in adipose tissue for oxidation. This is done by increasing the adipose cAMP concentration. The cAMP allosterically activates cAMP-dependent protein kinase which, in turn, increases the phosphorylation levels of susceptible enzymes including HSL. The phosphorylation activates HSL, thereby stimulating lipolysis in adipose tissue and resulting in the release of fatty acids into blood stream (Figure 4.5) (Salway, 1994). On the other hand, glucagon-stimulated cAMP-dependent protein kinase also inhibits the fatty acid synthesis by inactivating ACC which is the rate-limiting enzyme in fatty acid synthesis. Furthermore, as a long term adaptation to prolonged fasting, cortisol is secreted by the adrenal cortex. It stimulates the synthesis of HSL, thereby resulting in more fatty acids being mobilized from the adipose reserves for oxidation to provide energy (Salway, 1994). In contrast, cortisol decreases the amount of LPL, so that the rate of fatty acid storage becomes minimum and may even be halted.

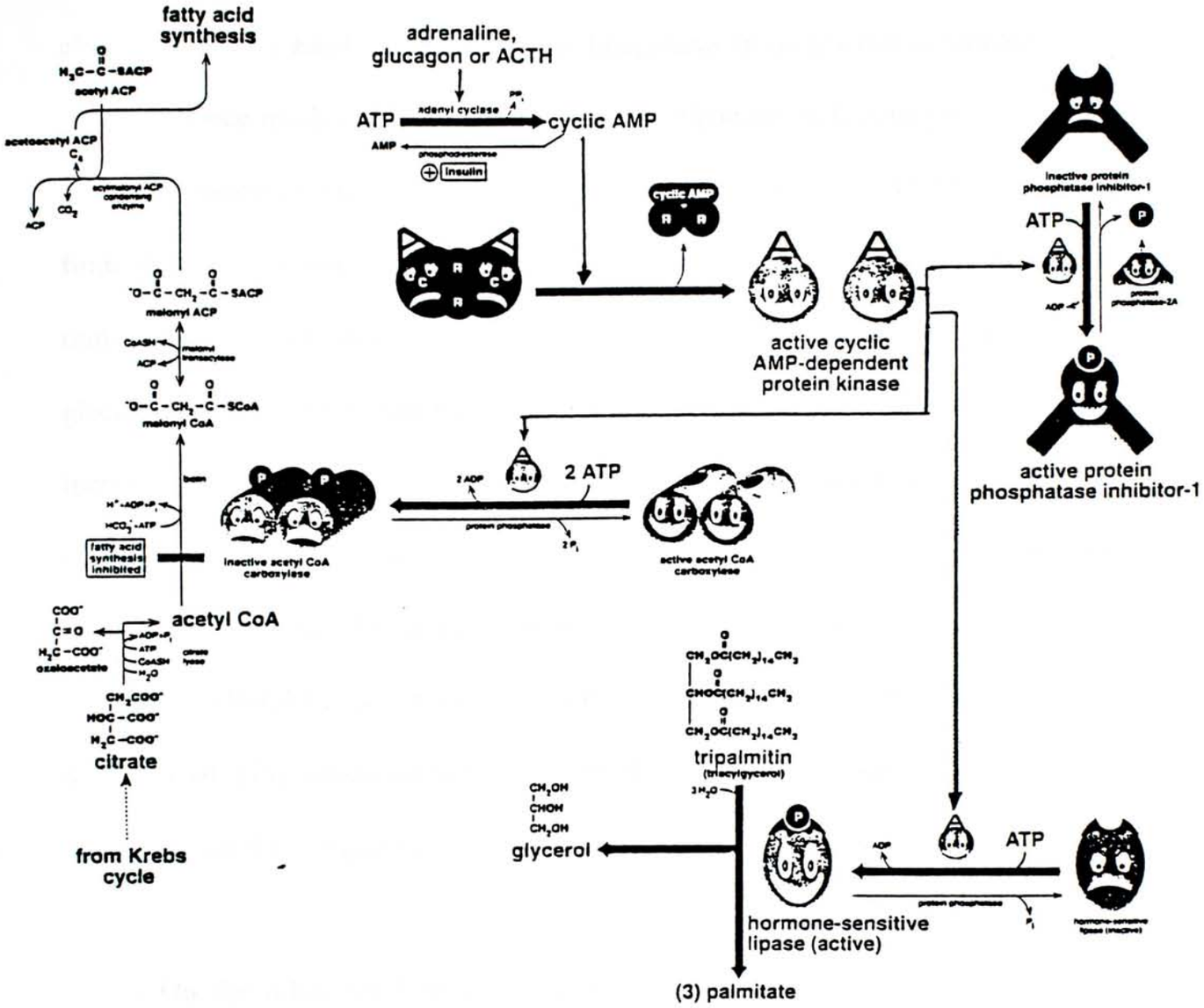


Figure 4.5 Hormonal regulation of mobilization of fatty acids from adipose tissue (Salway, 1994).

4.1.2.2 Fatty Acid Metabolism During Fed-State

Insulin is secreted by the pancreatic β-cells in response to an increase of blood glucose concentration in the fed-state. The actions of insulin counteract those of glucagon. On one hand, insulin increases lipogenesis from glucose in adipose tissue and liver. Once insulin binds to its receptors on adipocytes or hepatocytes, the rate of glucose transport into the cells is increased by 30-fold (Salway, 1994). This is resulted from the insulin-stimulated translocation and recyling of a latent pool of glucose transporter GLU T4 (Bernlohr and Simpson, 1996). This facilitates the transport of glucose into the cytosol where it is metabolized to triglycerides. In addition, insulin increases lipogenesis by stimulating the synthesis of the important lipogenic enzymes including FAS, PK, ACC and ME (Goodridge, 1987; Custodia *et al.,* 1994). Besides, insulin also promotes the uptake and storage of fatty acids from blood stream into adipocytes (Bernlohr and Simpson, 1996). This is achieved by stimulating the synthesis of LPL which initiate the entry of lipoprotein-packaged fatty acids into adipose tissue for storage (Bensadoun, 1991).

On the other hand, insulin is also a potent inhibitor of the hydrolysis of triglycerides in adipose tissue (Salway, 1994). Besides inhibiting HSL activity, insulin can also efficiently reduce the number of adipocyte β-adrenergic receptor which mediate the lipolytic responses (Bernlohr and Simpson, 1996). Hence, the release of fatty acids from adipose tissue is inhibited. As the effects of glucagon and insulin counteract each other, the glucagon-insulin ratio is therefore of prime importance in determining the rate and direction of fatty acid metabolism.

4.2 Objective of the Present Study

In the previous study, we demonstrated that weight cycling altered the fatty acid composition in carcass and adipose tissue with elevated proportion of SFAs and depletion of 18:2n-6 and 18:3n-3 . Furthermore, weight cycling with high-fat diet was shown to induce the enlargement of adipocytes and form heavier fat pads. These alterations are proposed to be closely related to the metabolic adaptation with increased lipogenesis. Moreover, insulin is associated with the energy expenditure and reservation during fasting and feeding.

Therefore, the objective of this study was to analyze the activities of enzymes (LPL, FAS, ME, ACC, PK and PEPCK) which are important in the fatty acid synthesis and storage. The levels of plasma insulin and plasma glucagon during weight cycling were also measured in order to explain the observations we found in previous studies.

4.3 Materials and Methods

4.3.1 Samples

All the samples including liver, adipose tissue and serum were obtained in the previous study in Chapter 3. The rats were divided into two main groups: HF and MF groups. The rats in each group were sub-divided into WC rats and CTL rats. The WC rats were under 2 weight cycles of 40% food restriction for 7 days and then followed which 10 days of *ad libitum* refeeding. The rats were killed at interval as mentioned in Section 3.3.1, Chapter 3. The serum was separated from whole blood by centrifugation (2000 g for 15 min). Liver and adipose tissue were washed with chilled saline and freeze-clamped in liquid nitrogen immediately after removal from the rats. Serum, liver and adipose tissue were stored in aliquots at -76 °C for the analysis of enzymatic activities and determination of serum insulin and glucagon levels.

4.3.2 Enzymatic Analysis

The activities of the enzymes determined in this study were: LPL, FAS, ME, PK, ACC and PEPCK.

4.3.2.1 Lipoprotein Lipase (LPL; EC 3.1.1.34)

The activity of LPL was determined as described by Nilsson-Ehle and Schotz (1976) with some modifications.

4.3.2.1.1 Substrate Preparation

(I) Purification of Tri-[9,10-^{3}H] oleoylglycerol ([^{3}H]TO)

Ten mCi of [^{3}H]TO (414 mCi/mmole, Amersham) in petroleum ether (b.p. 30-60 °C) with 50mg unlabeled TO (Sigma) as carrier was applied to a column (5 ml syringe) of 2 g silicic acid (Sigma) covered by a layer of 200 mg of florisil (Sigma) to improve the separation of free fatty acids. It was then washed with 50 ml of petroleum ether. TO was finally eluted with 5 % (v/v) diethyl ether in petroleum ether (80-90% recovery).

(II) Preparation of Concentrated Substrate

The eluent obtained in (I) was flushed with nitrogen gas to evaporate the solvent. The labeled and unlabeled TO were further mixed with 550 mg of unlabeled TO and 36 mg of lecithin (Sigma) in chloroform. The mixture was then dried under a stream of nitrogen. The dried lipids were emulsified in 10 ml (12.5 g) of glycerol by homogenization for 5 min continuously using a Polytron in ice-water bath. Finally, a concentrated substrate was prepared, stored at 4 °C and used within 2 weeks.

(III) Preparation of Assay Substrate

Substrate solutions for assay were prepared freshly by dilution of 1 volume of concentrated substrate with 4 volume of 0.2 M Tris-HCl buffer (pH 8.0) containing 3% (w/v) BSA (Sigma) and 1 volume of cold fasting serum (pre-heated at 62 °C for 10 min to eliminate any endogenous lipolytic activity). The mixture was then shaken vigorously on a Vortex mixer for 5 sec. The opague emulsion obtained was used within 2 h (including the time taken for the enzymatic reaction).

4.3.2.1.2 Preparation of sample

Epididymal (perirenal) adipose tissue (300 mg) was homogenized in 150 µl medium containing cold fasting serum (pre-heated at 62 °C for 10 min) : 67 mM Tris-HCl (pH 7.4), 1:3 (v/v) using glass Potter-Elvehjem homogenizer with Teflon pestle for 1 min. After adding 3 ml of cold acetone, the tissue was re-homogenized and centrifuged (12,000 g for 2 min). The sediment was then homogenized and extracted for thrice (twice with the same volume of cold acetone and once with cold ether; 12,000g for 2 min). The powder containing LPL and, a membrane bound enzyme, was dried under a stream of nitrogen gas. The powder extracted was mixed with 1.5 ml of buffer (50 mM Tris-HCl, pH 8.0; 1 M ethylene glycol). The supernate (in which all the LPL was dissolved) was saved after centrifugation (40,000 g for 30 min) and was employed as the enzyme source.

4.3.1.3 Enzyme Assay

The reaction mixture (0.2 ml) was prepared by adding 0.1 ml of freshly prepared substrate to 0.1 ml of enzyme such that the final concentration was as follows : TO (5.66 µmoles/ml), lecithin (0.35 µmoles/ml), BSA (1%, w/v), serum (8.5%, v/v in 70 mM Tris-HCl, pH 8.0 containing 8.5% glycerol). The mixture was then incubated at 37 °C for 30 min. The reaction was stopped by adding 3.25 ml of methanol : chloroform : heptane (1.41 : 1.25 : 1) and 1.05 ml of 0.1 M potassium carbonate-borate buffer (pH 10.5) followed by vortexing for 15 sec and centrifugation (3,000 g for 25 min). Finally, 1 ml of methanol-water phase was counted for radioactivity using 15 ml scintillation fluid (Triton-X 100 : Toluene, 1:1, v/v) containing 0.4% PPO (w/v, Sigma) and 0.04% POPOP (w/v, Sigma). A series of

controls was also performed without adding the enzyme solutions. LPL activity (mU) was expressed as the amount of enzyme needed to release of 1 nanomole of oleic acid per min at 37 °C.

4.3.2.2 Fatty Acid Synthase (FAS; EC 2.3.1.85)

The activity of FAS was measured according to the method of Nepokroeff *et al.* (1975). One g of liver (or 2 g of adipose tissue) was homogenized in 1.5 volumes of homogenizing buffer (70 mM $KHCO_3$; 85 mM K_2HPO_4; 9 mM KH_2PO_4; 1 mM DTT; pH 8.0) using Polytron in an ice-water bath. The homogenate was centrifuged at 20,000 g for 10 min. The supernatant was centrifuged again at 105,000 g for 60 min. The supernatant obtained was immediately used for the enzyme assay and the activity of FAS was measured spectrophotometrically by monitoring the rate of NADPH oxidation. The reaction mixture (500 μmoles potassium phosphate buffer, pH 7.0; 33 nmoles acetyl-CoA; 100 nmoles malonyl-CoA; 100 nmoles NADPH; 1 μmole β-mercaptoethanol) was pre-incubated at 30 °C for 5 min. The reaction was initiated by the addition of 50-100 μg of the enzyme protein (supernatant after centrifugation at 105,000 g), which has been pre-incubated in 40 μl of activating buffer (1 M potassium phosphate, pH 7.0; 10 mM DTT) at 37 °C for 15 min, into 960 μl of the pre-incubated reaction mixture. The oxidation of NADPH was followed at 340 nm. A correction was made for the rate of NADPH oxidation in the absence of malonyl-CoA as a background. One unit of enzyme activity represented the amount of enzyme needed to synthesize 1 nanomole of palmitic acid (equivalent to the oxidation of 14 nmoles of NADPH) per min.

4.3.2.3 Malic Enzyme (ME; EC 1.1.1.40)

ME activity was estimated from the rate of NADPH formation at 25 °C (Hsu and Lardy, 1969). One g of liver was first washed in 0.25 M sucrose and cut into small pieces. It was then homogenized in 3 volumes of 0.25 M sucrose using glass Potter-Elvehjem homogenizer in ice-water bath. The homogenate was centrifuged at 105,000 g for 1 h to obtain the supernatant. The reaction was initiated by mixing 990 µl of assay buffer (400 mM triethanolamine-HCl : 30 mM L-malate : 120 mM $MnCl_2 \cdot 4H_2O$: 3.4 mM NADP, 10:1:2:4, v/v/v/v, pH 7.4) with 10 µl enzyme (105,000g supernatant). The reduction of $NADP^+$ was followed at 340 nm The background activity was measured in the absence of L-malate. One unit of enzyme activity was defined as the amount of enzyme needed to reduce 1 nanomole of $NADP^+$ per min at 25 °C.

4.3.2.4 Pyruvate Kinase (PK; EC 2.7.1.40)

The activity of pyruvate kinase was measured by the rate of NADH reduction spectrophotometrically as described by Imamura and Tanaka (1972). Liver (1g) was homogenized in 3 volumes of homogenization buffer (20 mM Tris-Cl, pH 7.5; 100 mM KCl; 5 mM $MgSO_4$; 1 mM EDTA; 0.2 mM Fru-1,6-P_2; 10 mM β-mercaptoethanol) using Polytron for 1 min in ice-water bath and the supernatant was obtained by centrifugation at 20,000 g for 1 h. The enzymatic reaction was initiated by adding 10 µl supernatant into the assay buffer (50 mM Tris-Cl, pH 7.5; 0.1 M KCl; 5 mM Mg SO_4; 2 mM PEP; 0.5 mM Fru-1,6-P_2; 0.18 mM NADH; 8 units of lactate dehydrogenase) which was pre-incubated at 37 °C. The oxidation of NADH was followed at 340 nm spectrophotometrically. One unit of enzyme activity was defined

as the amount of enzyme catalyzing the formation of 1 μmole of pyruvate (or oxidizing 1 μmole of NADH in the coupled system) per min under the assay conditions. Background was measured by starting the assay without PEP.

4.3.2.5 Acetyl-CoA Carboxylase (ACC; EC 6.4.1.2)

The method of Inone and Lowenstein (1975) was used to determine the activity of ACC. In brief, 2 g of liver was homogenized in 2 volumes of buffer (0.05 M Tris-Cl, pH 7.5; 20 mM sodium citrate; 0.5 mM EDTA; 5 mM β-mercaptoethanol) using Polytron for 10 sec in ice-water bath. It was centrifuged at 2,000 g for 10 min and the residue was homogenized again with 1 ml of the same buffer using a glass Potter-Elvehjem homogenizer. The homogenate was combined with the supernatant from the first centrifugation and re-centrifuged at 14,000 g for 45 min. The residue was then washed with 1 ml of buffer. The washing was combined with the supernatant from the second centrifugation and then centrifuged again at 105,000 g for 45 min to obtain the supernatant.

Four ml of the supernatant was loaded to a Sephadex G-25 column (2.25x30 cm, Amersham) equilibrated with 20 mM Tris-Cl (pH 7.5) containing 1 mM DTT. Fractions of 1 ml were collected and the fractions with the highest protein concentration were pooled.

The crude enzyme (supernate) was pre-activated by mixing 0.5 ml of the fraction (crude enzyme) with 0.5 ml of activating buffer (20 mM sodium citrate; 20 mM $MgCl_2$; 1 mM DTT; 50 mM Tris-Cl, pH 7.5) containing 0.5 mg/ml of BSA (fatty

acid poor or free, Sigma) at 37 °C for 30 min. The activated enzyme was used for assay within 20 min. The assay was initiated by adding 30 µl of the activated enzyme into 370 µl of assay buffer (100 mM Tris-Cl, pH 7.5; 1 mM DTT; 0.2 mM acetyl-CoA; 20 mM sodium bicarbonate ($NaH^{14}CO_3$) (0.25µCi/µmole, Amersham); 5 mM ATP; 20 mM citrate; 20 mM $MgCl_2$ and 0.5 mg/ml BSA) and the reaction mixture was incubated at 37 °C for 5 min. The reaction was stopped by adding 0.1 ml of 4 N HCl and the unreacted $^{14}CO_2$ was expelled by a gentle stream of air. The residue was then dissolved in 1 ml of H_2O and its radioactivity was counted. One unit of ACC activity was equal to the amount of enzyme needed to form 1 µmole of malonyl-CoA at 37 °C per min.

4.3.2.6 Phosphoenolpyruvate Carboxykinase (PEPCK, EC 4.1.1.32)

The activity of PEPCK was measured by coupling the reaction with NADH oxidation in the presence of excess malate dehydrogenase according to Petrescu *et al.* (1979). Firstly, the liver cytosol was prepared as described in malic enzyme analysis (Section 4.3.2.3). The reaction was initiated by adding 20 µl of 0.2 mM dGDP to a reaction mixture containing 960 µl assay buffer (50 mM Tris-Cl, pH 7.4; 20 mM $NaH^{14}CO_3$ (saturated with CO_2); 0.5 mM PEP; 1 mM $MnCl_2$; 0.1 mM NADH; 2 units of L-malate dehydrogenase) and 20 µl of liver homogenate. The reaction was followed at 340 nm in a spectrophotometer and a correction without bicarbonate was made as a control. One unit of PEPCK activity was defined as the amount of enzyme needed to oxidize 1 nanomole of NADH per min.

4.3.2.7 Determination of Protein Content

The specific activity of the enzyme was expressed as the activity defined for particular enzyme per mg of protein, i.e. U/mg. The total protein concentration was measured by the method of Bradford (1976) with BSA as standard.

4.3.3 Determination of Serum Insulin and Serum Glucagon

The level of serum insulin and glucagon was determined by using I-125 Radioimmunoassay (RIA) kits (Amersham and Linco, respectively).

4.3.4 Statistics

All the measurements were repeated two to three times. Data were pooled and expressed as means ± SD. Differences between the CTL and WC rats were analyzed by analysis of variance (ANOVA) using SigmaStat Advisory Statistical Software (SigmaStat versiom 2.01, SPSS Inc., Chicago, lL, USA) and only when $p<0.05$ was considered as significant.

4.4 Results

4.4.1 Enzymatic Analysis

4.4.1.1 Lipoprotein Lipase

There was a general increasing trend for the specific enzymatic activity for LPL of all the rats in both the HF and MF groups. In the HF group, the LPL activity of the WC rats decreased during the fasting periods of the two cycles. During the refeeding, the activity increased rapidly, and then remained significantly higher than that of the CTL rats throughout the periods. At the end of the two WCs, the LPL activity of the WC rats was essentially higher than that of the CTL rats. Similar results were obtained for both epididymal and perirenal adipose tissue (Figures 4.6 and 4.7).

In the MF group, the LPL activity of the WC rats also decreased and then increased simultaneously with the body weight during the weight cycles. However, at the end of the two cycles, the LPL activity of the WC rats was not significantly different from that of the CTL rats. Similar results were also obtained for both epididymal and perirenal adipose fat pads (Figures 4.8 and 4. 9).

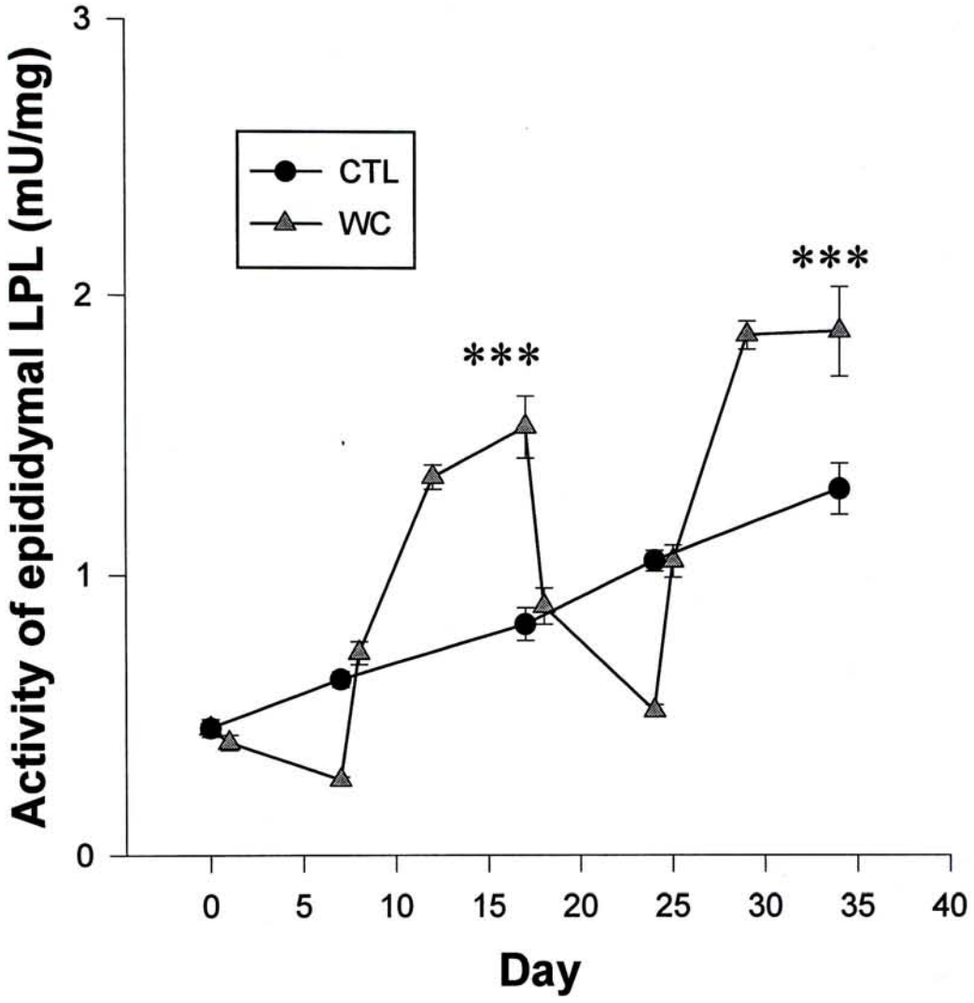


Figure 4.6 Specific activity of epididymal LPL of rats fed HF-diet. (***p<0.001, difference between CTL and WC rats.)

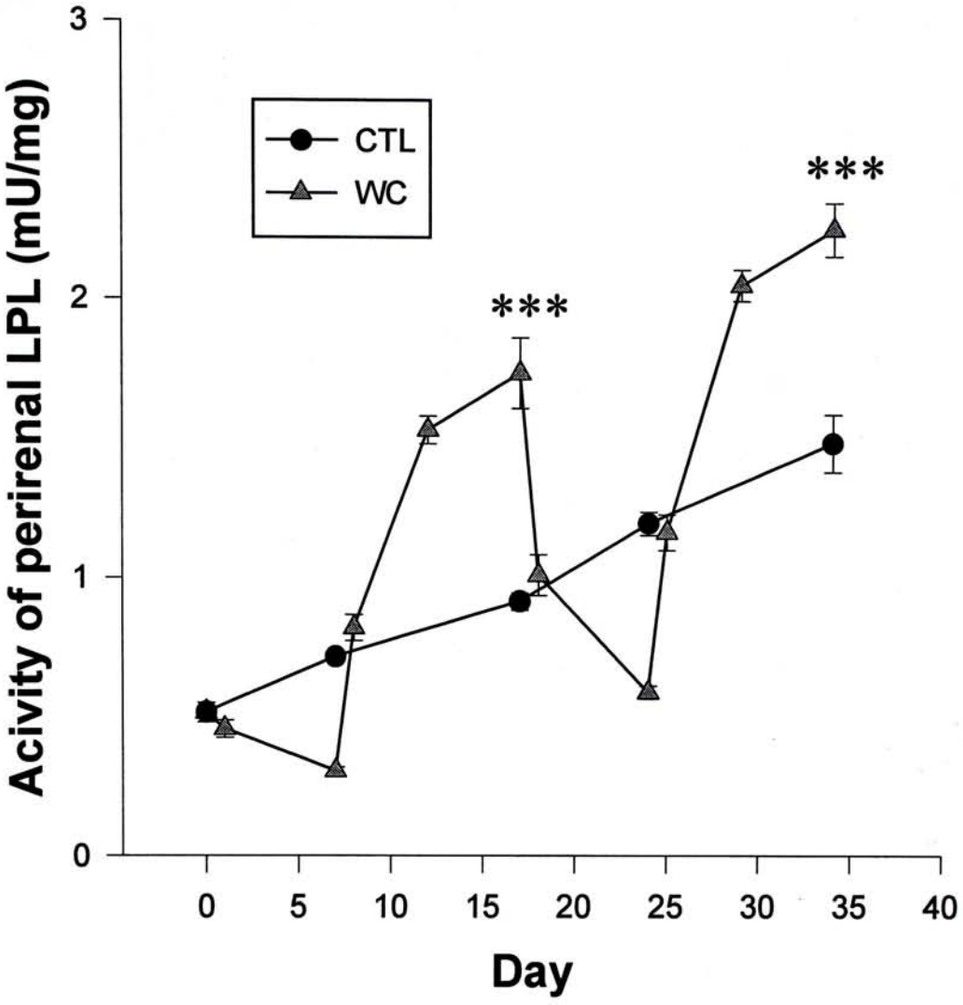


Figure 4.7 Specific activity of perirenal LPL of rats fed HF-diet. (***p<0.001, difference between CTL and WC rats.)

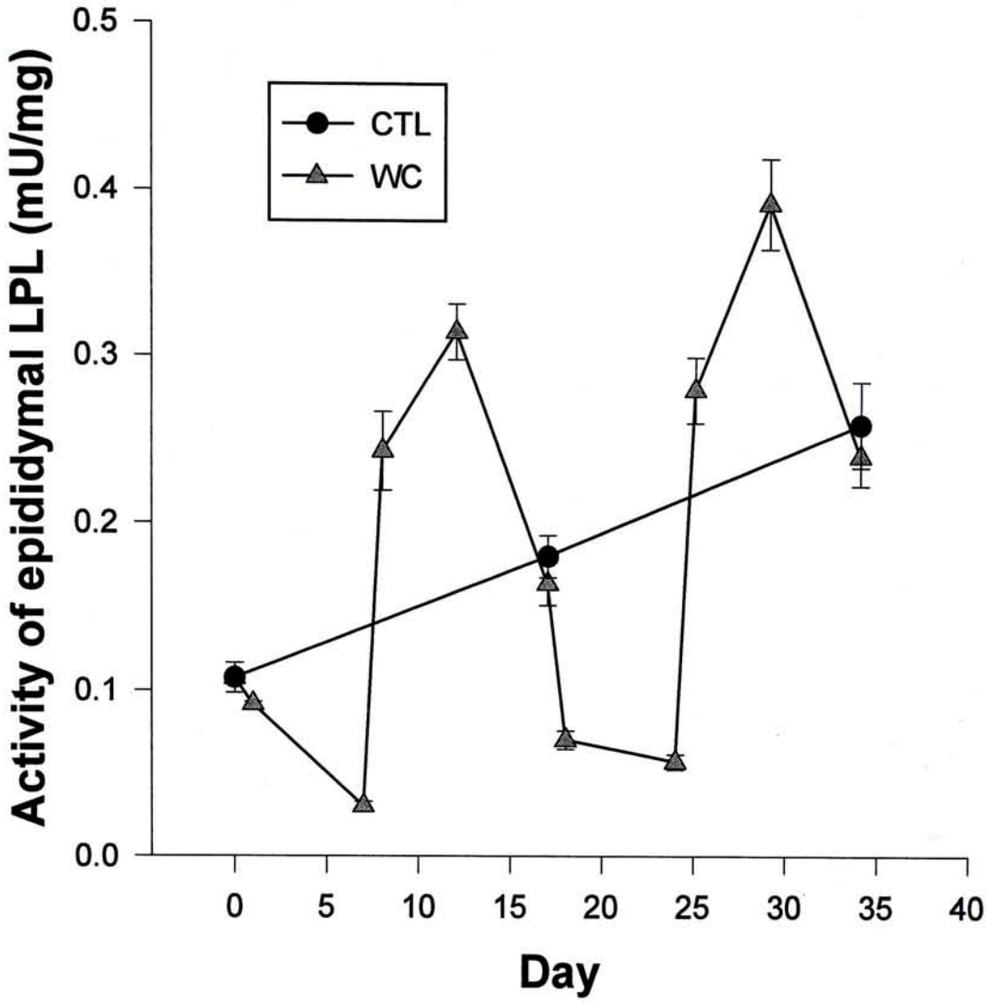


Figure 4.8 Specific activity of epididymal LPL of rats fed MF-diet.

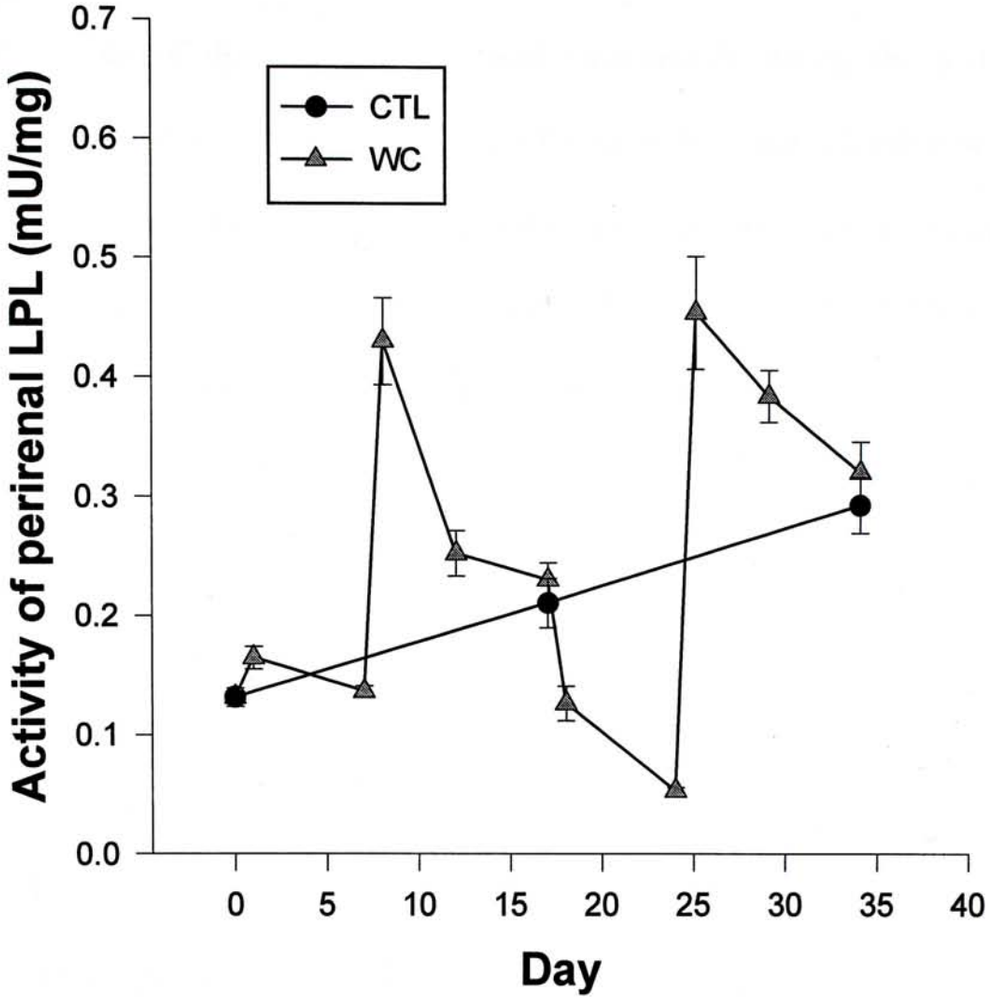


Figure 4.9 Specific activity of perirenal LPL of rats fed MF-diet.

4.4.1.2 Fatty Acid Synthase

In the HF group, the activities of FAS from the epididymal and perirenal adipose tissues of the WC rats decreased considerably during the partial energy restriction, but with an overshoot in activities throughout the refeeding periods. The FAS activity of the WC rats at the end of the two cycles was also significantly higher than that of the CTL rats (Figures 4.10 and 4.11). For the hepatic FAS, the specific activity of the CTL rats remained unchanged during refeeding (Figure 4.12). Although the final FAS activity of the WC rats was significantly different from that of the CTL rats, this difference was much smaller compared with the difference observed in adipose tissues.

In the MF group, the activity of adipose (epididymal and perirenal) FAS and hepatic FAS all showed similar responses to weight cycling (Figures 4.13, 4.14 and 4.15). During the energy restriction, their activities dropped dramatically. However, during the refeeding, their activities only rose to a level similar to that of the CTL rats. Moreover, the final FAS activities at the end of the two weight cycles were not significantly different between WC rats and CTL rats. In fact, the former was slightly lower than the latter.

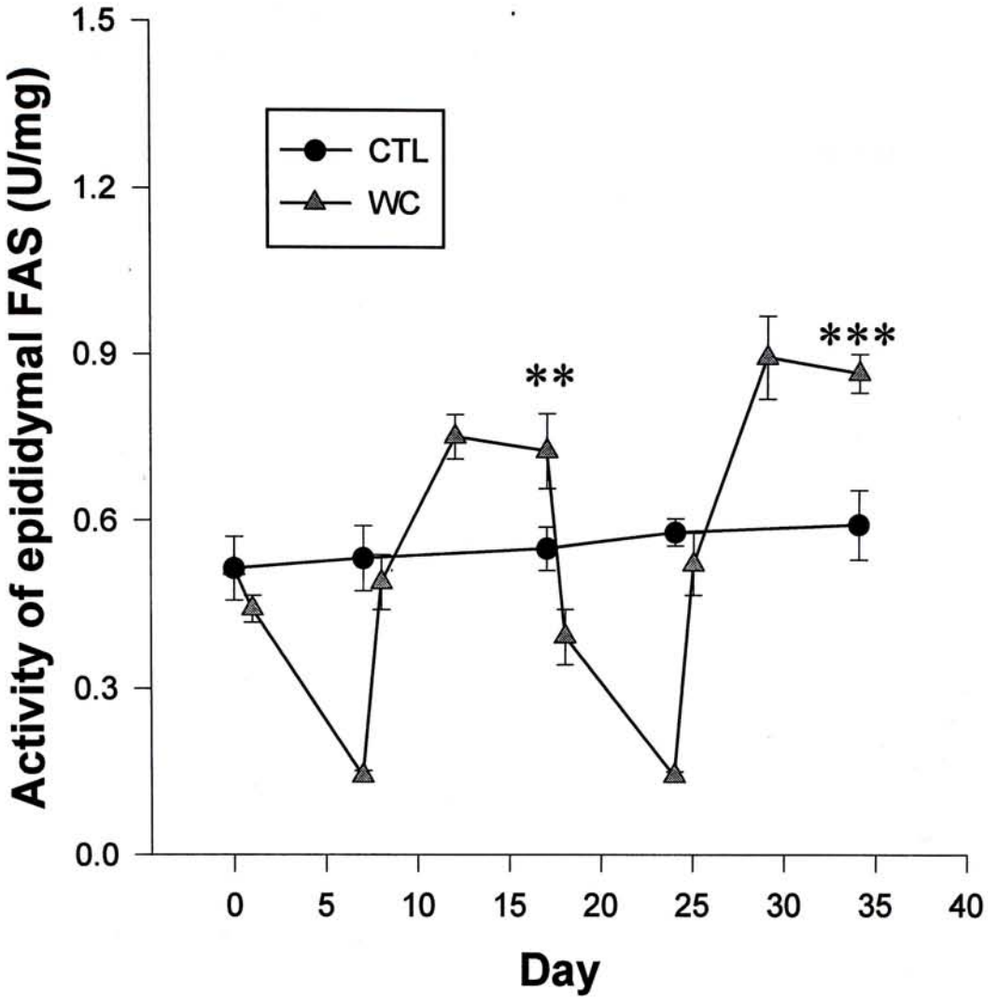


Figure 4.10 Specific activity of epididymal FAS of rats fed HF-diet. (**p<0.01 and ***p<0.001, difference between CTL and WC rats.)

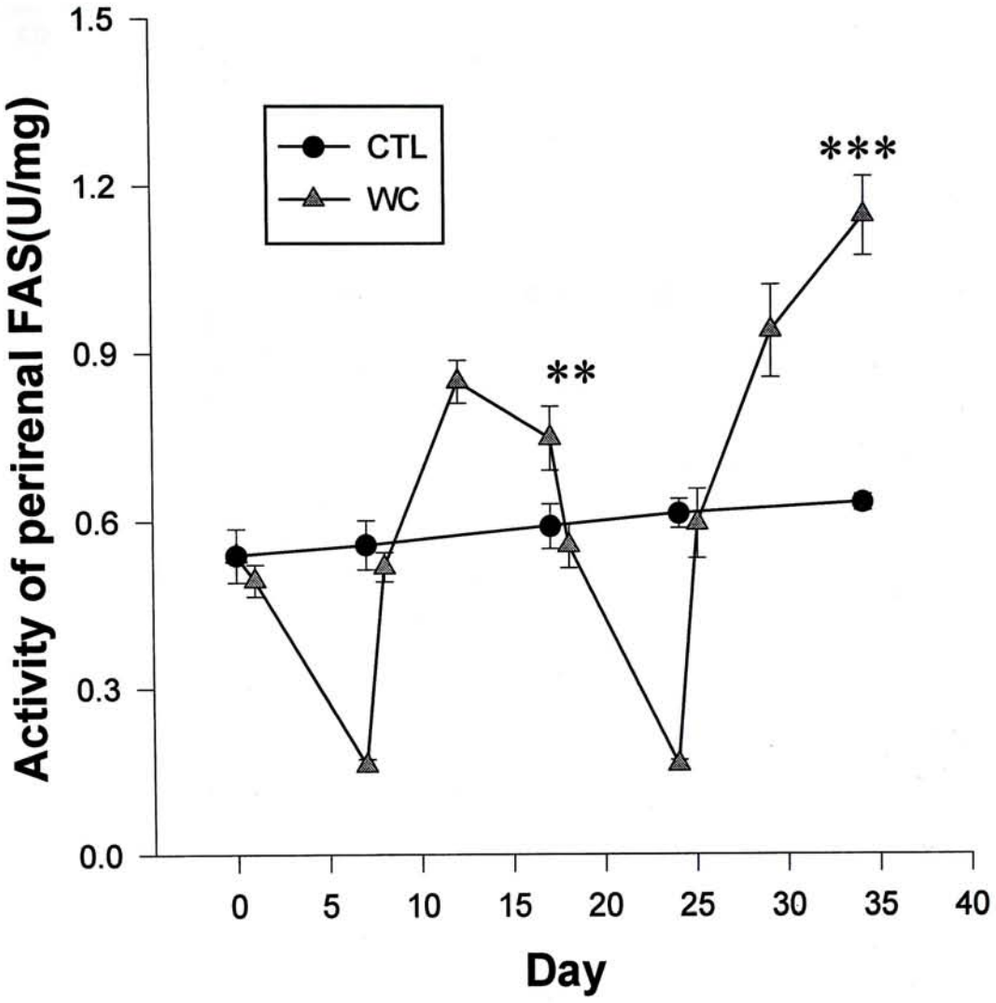


Figure 4.11 Specific activity of perirenal FAS of rats fed HF-diet. (**p<0.01 and ***p<0.001, difference between CTL and WC rats.)

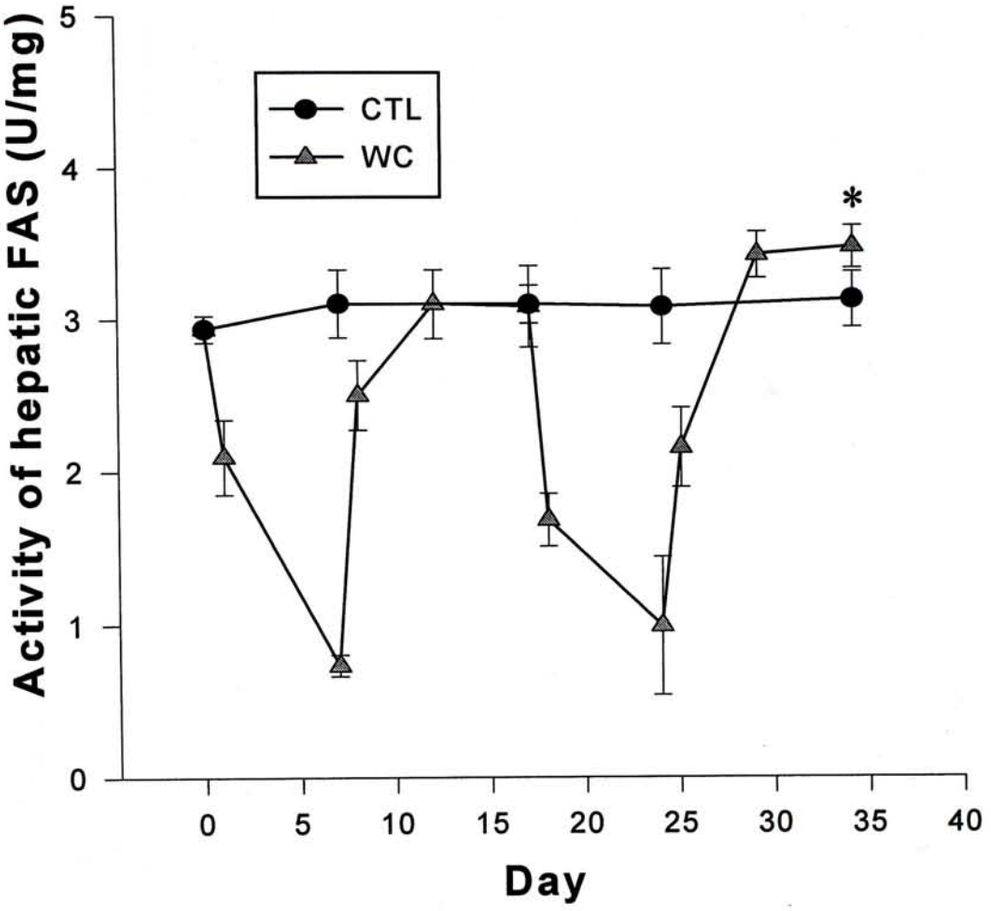


Figure 4.12 Specific activity of hepatic FAS of rats fed HF-diet. (*p<0.05. difference between CTL and WC rats.)

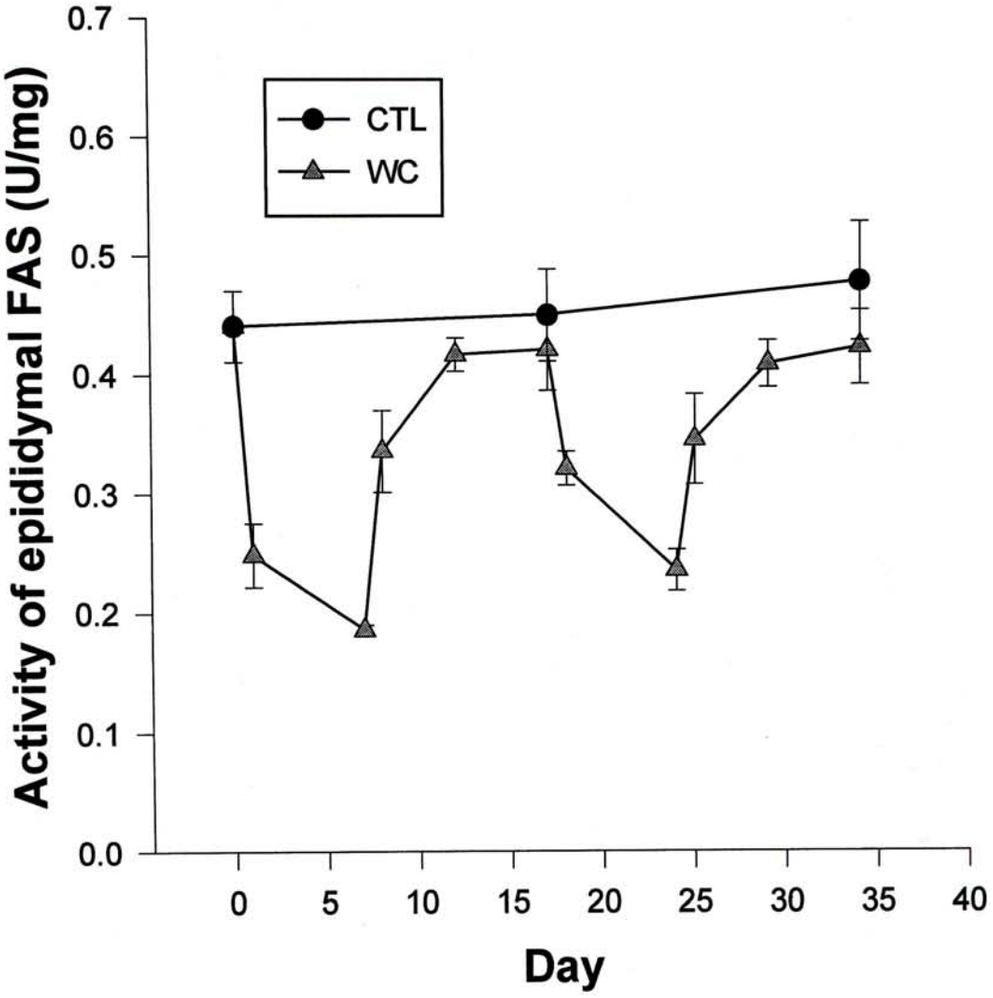


Figure 4.13 Specific activity of epididymal FAS of rats fed MF-diet.

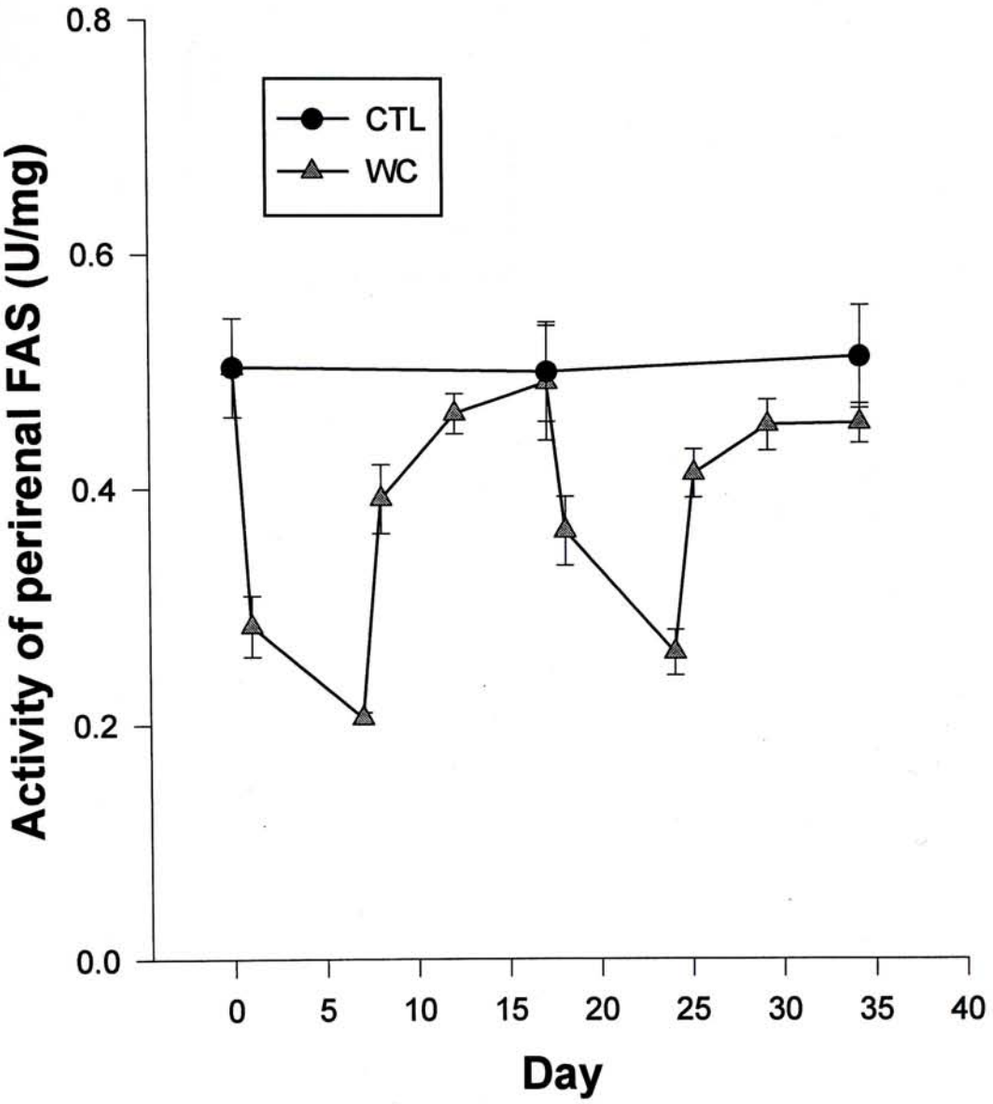


Figure 4.14 Specific activity of perirenal FAS of rats fed MF-diet.

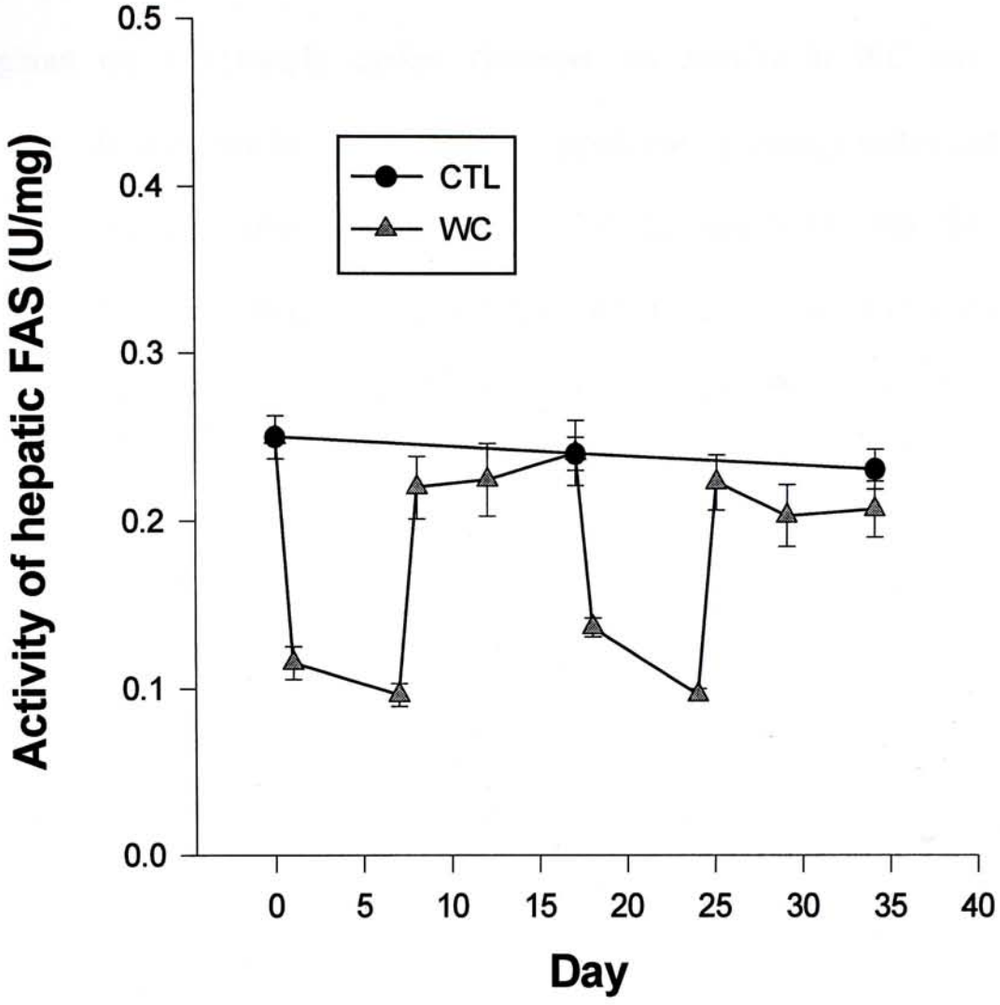


Figure 4.15 Specific activity of hepatic FAS of rats fed MF-diet.

4.4.1.3 Malic Enzyme

In the HF group, the activity of hepatic ME of the CTL rats remained constant throughout the two weight cycles. However, its activity in WC rats fluctuated coincidentally with the body weight. It dropped during energy restriction and rose rapidly during the refeeding. At the end of the two WCs, the WC rats had considerably higher activity than that in CTL rats (Figure 4.16). Furthermore, it was noteworthy that the difference of ME activity between CTL and WC rats was remarkably larger at the end of the second cycle than that of the first cycle.

In the MF group, the ME activity of the WC rats changed parallel to the body weight change. Distinct from the HF group, there was no significant difference in the final ME activity between the WC rats and CTL rats (Figure 4.17).

4.4.1.4 Pyruvate Kinase

The enzymatic activity of PK of the WC rats in the HF group decreased significantly during fasting, and then increased rapidly during the refeeding periods in both weight cycles. At the end of the cycles, the activity of the WC rats was slightly higher than that of the CTL rats (Figure 4.18).

In the MF group, the PK activity of the WC rats also decreased drastically during fasting, and with an overshoot in activity throughout the two refeeding periods. There was a significant difference in the final PK activity between the WC rats and

the CTL rats, with the WC rats having a higher PK activity than the CTL rats (Figure 4.19).

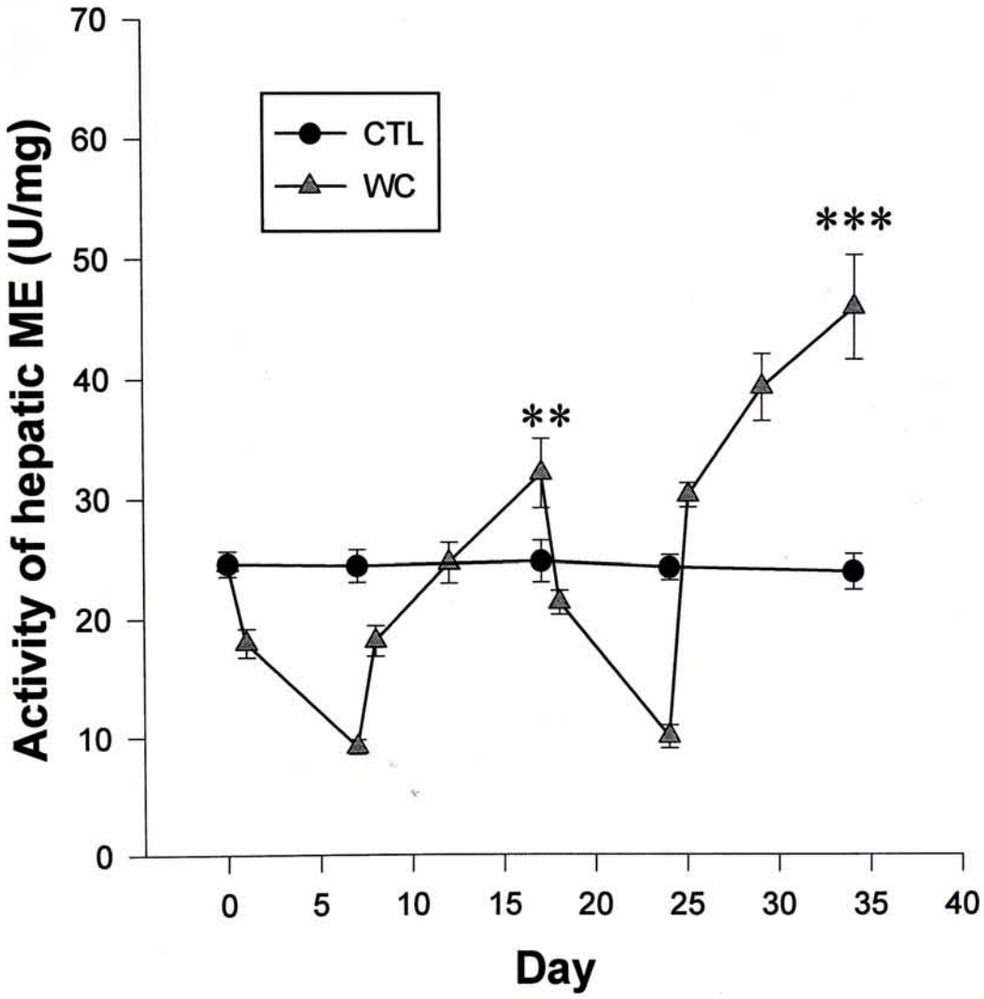


Figure 4.16 Specific activity of hepatic ME of rats fed HF-diet. (**p<0.01 and ***p<0.001, difference between CTL and WC rats.)

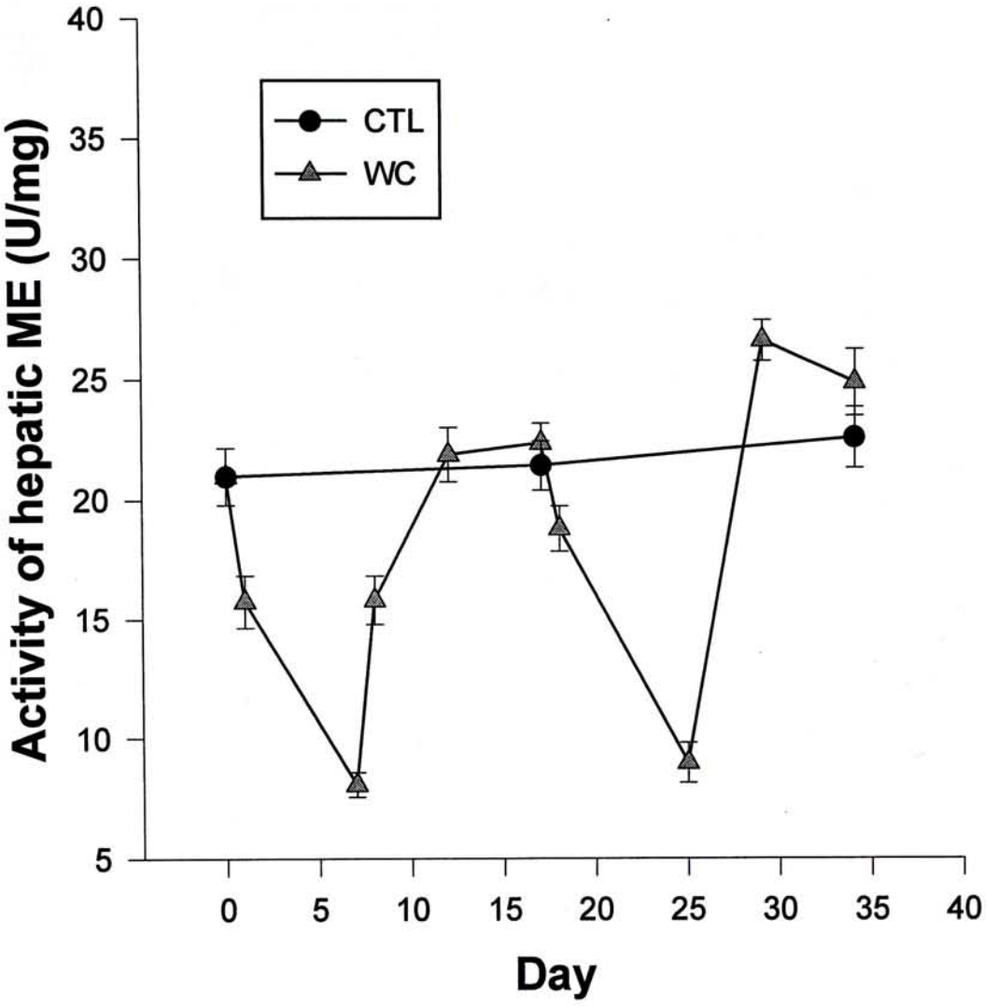


Figure 4.17 Specific activity of hepatic ME of rats fed MF-diet.

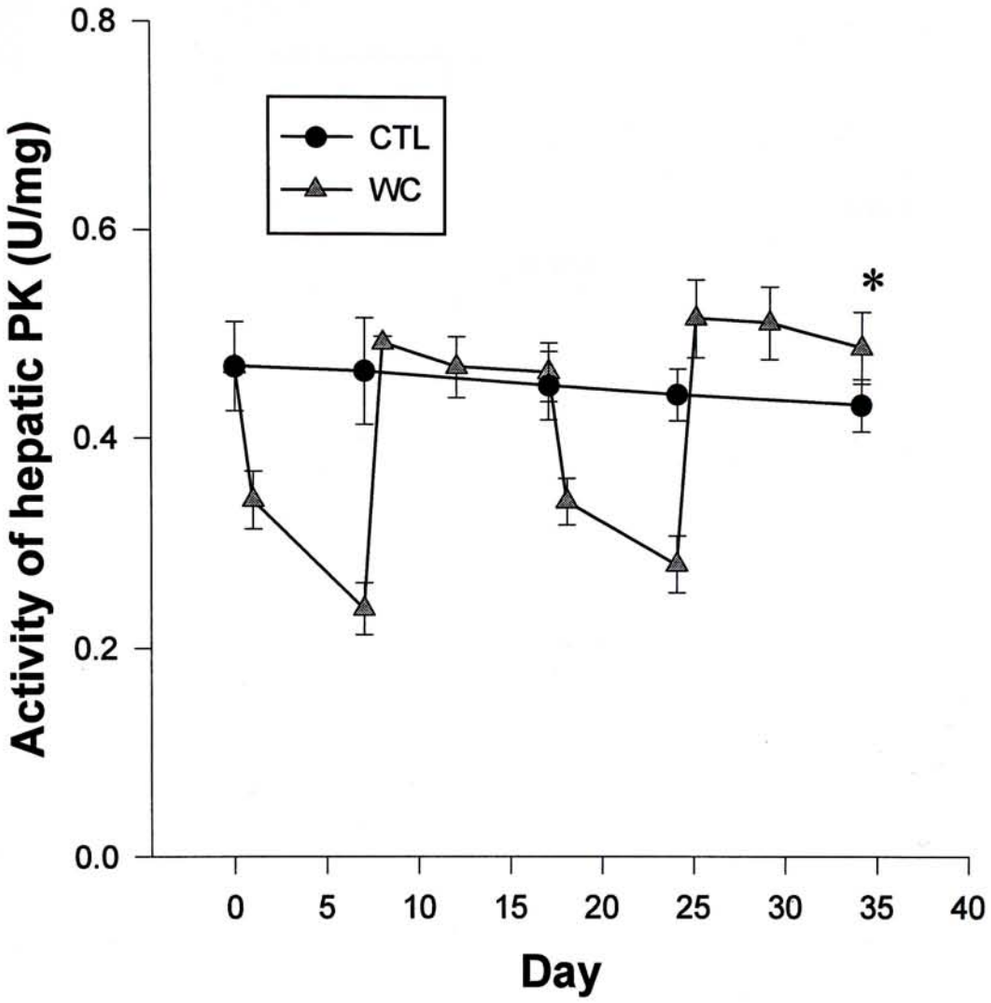


Figure 4.18 Specific activity of hepatic PK of rats fed HF-diet. (*p<0.05, difference between CTL and WC rats.)

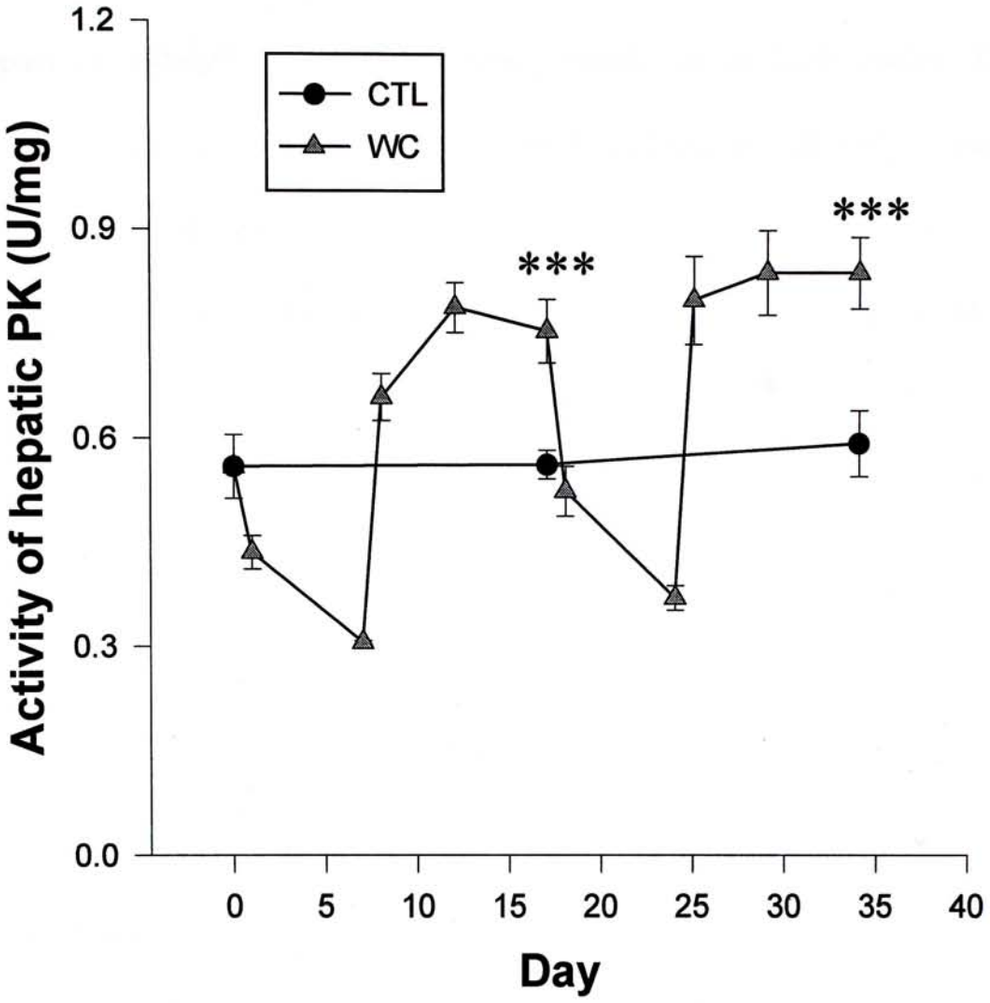


Figure 4.19 Specific activity of hepatic PK of rats fed MF-diet. (***p<0.001, difference between CTL and WC rats.)

4.4.1.5 Acetyl-CoA Carboxylase

The specific activity of ACC in the liver of the WC rats in the HF group decreased considerably during the energy restriction in both cycles. During the refeeding periods, the ACC activity rose rapidly and was significantly higher than that of the CTL rats (Figure 4.20).

In the MF group, the ACC activity of the WC rats also dropped when the rats were fasted. However, during the refeeding periods, its ACC activity was only slightly higher than that of the CTL rats, and there was no significant difference between the WC and CTL rats (Figure 4.21).

4.4.1.6 Phosphoenolpyruvate Carboxykinase

The activity of PEPCK of the WC rats in both HF and MF groups changed by a much smaller extent though it fluctuated coincidentally with their body weight. Different from the other enzymes, no matter it was HF or MF group, the PEPCK activity of the WC rats remained similar to that of the corresponding CTL rats throughout the two weight cycles (Figures 4.22 and 4.23).

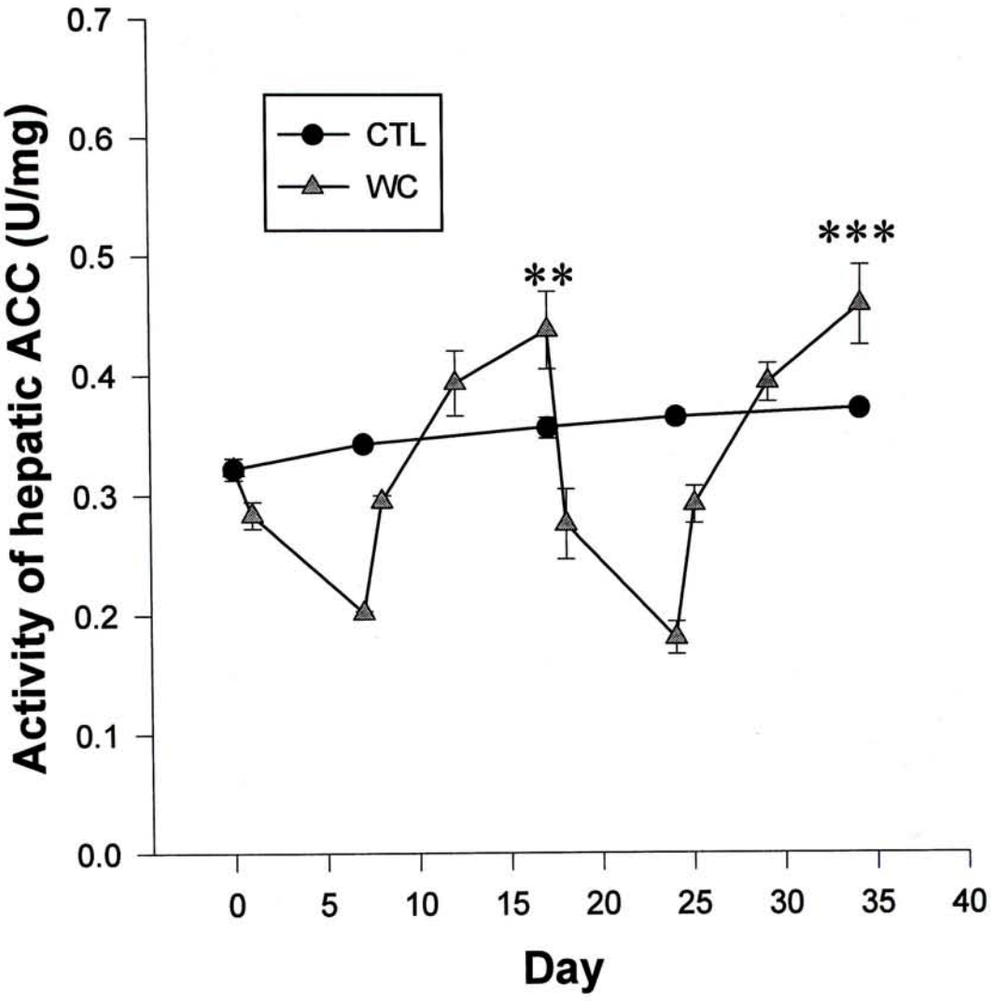


Figure 4.20 Specific activity of hepatic ACC of rats fed HF-diet. (**p<0.01 and ***p<0.001, difference between CTL and WC rats.)

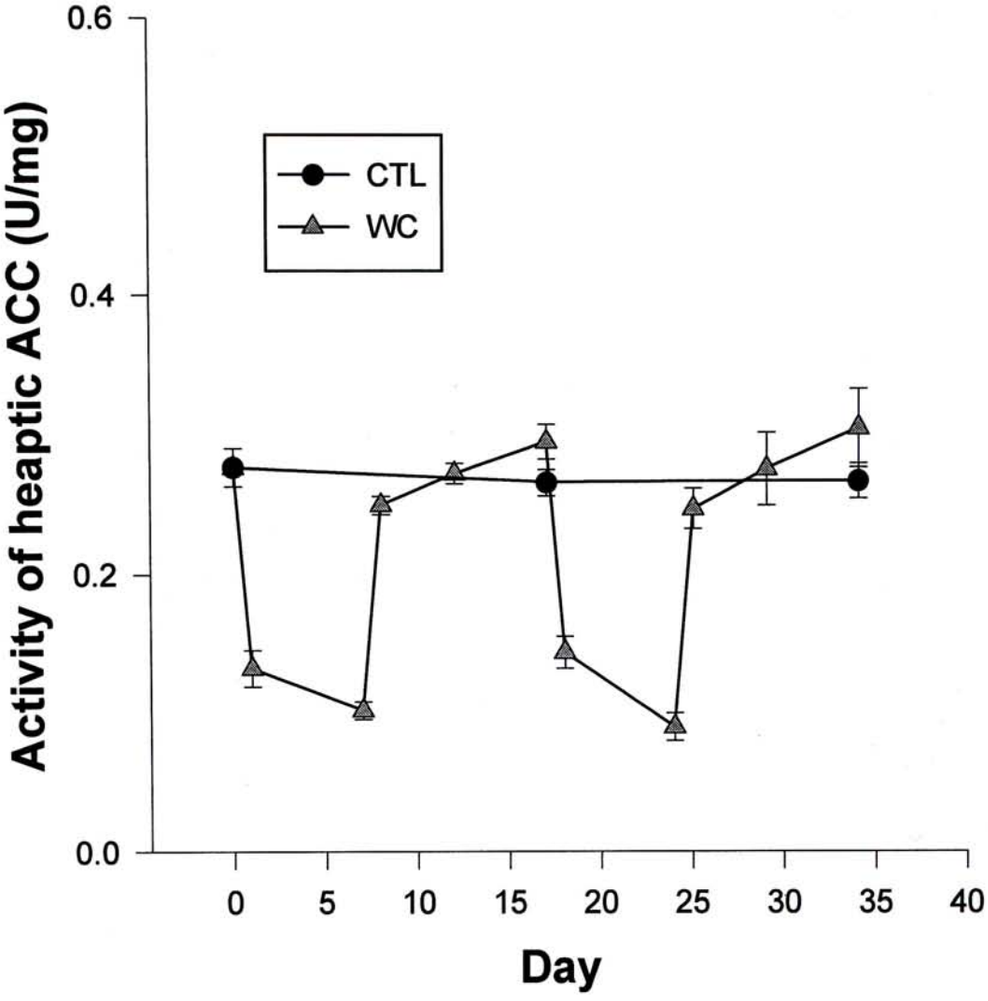


Figure 4.21 Specific activity of hepatic ACC of rats fed MF-diet.

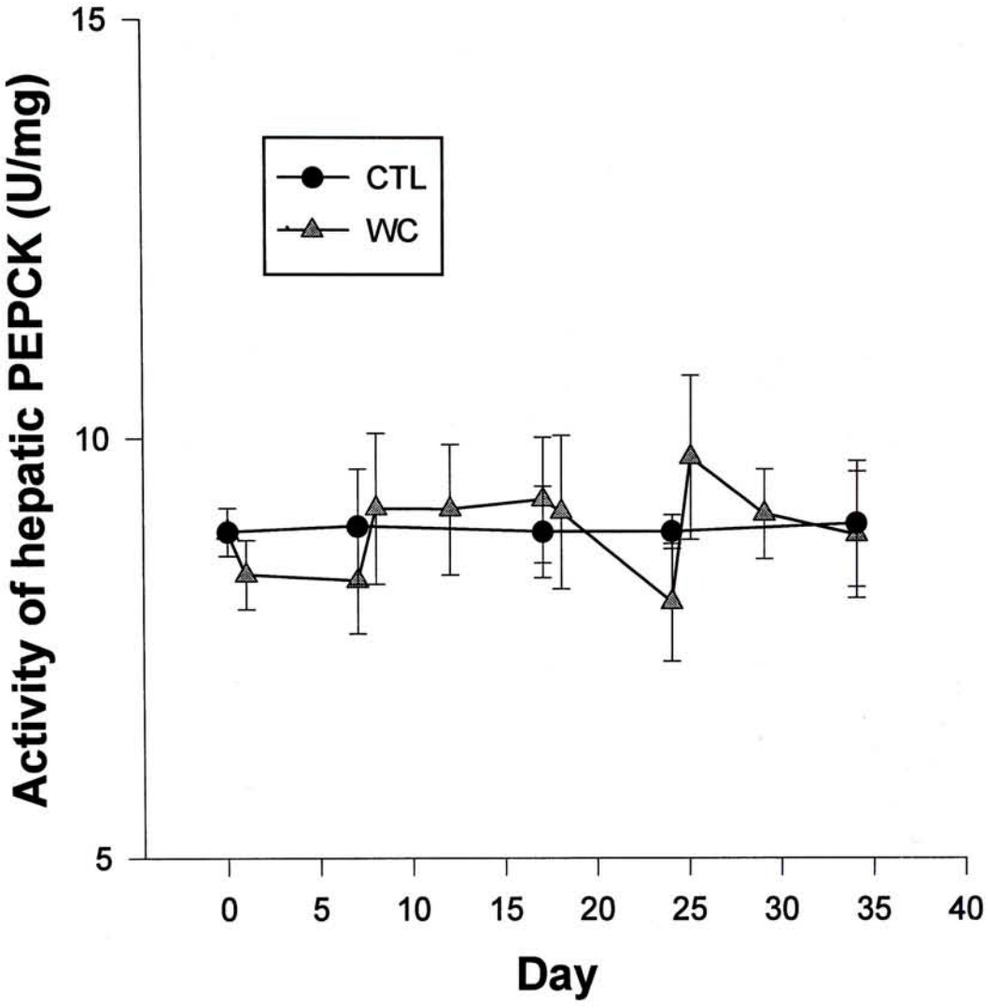


Figure 4.22 Specific activity of hepatic PEPCK of rats fed HF-diet.

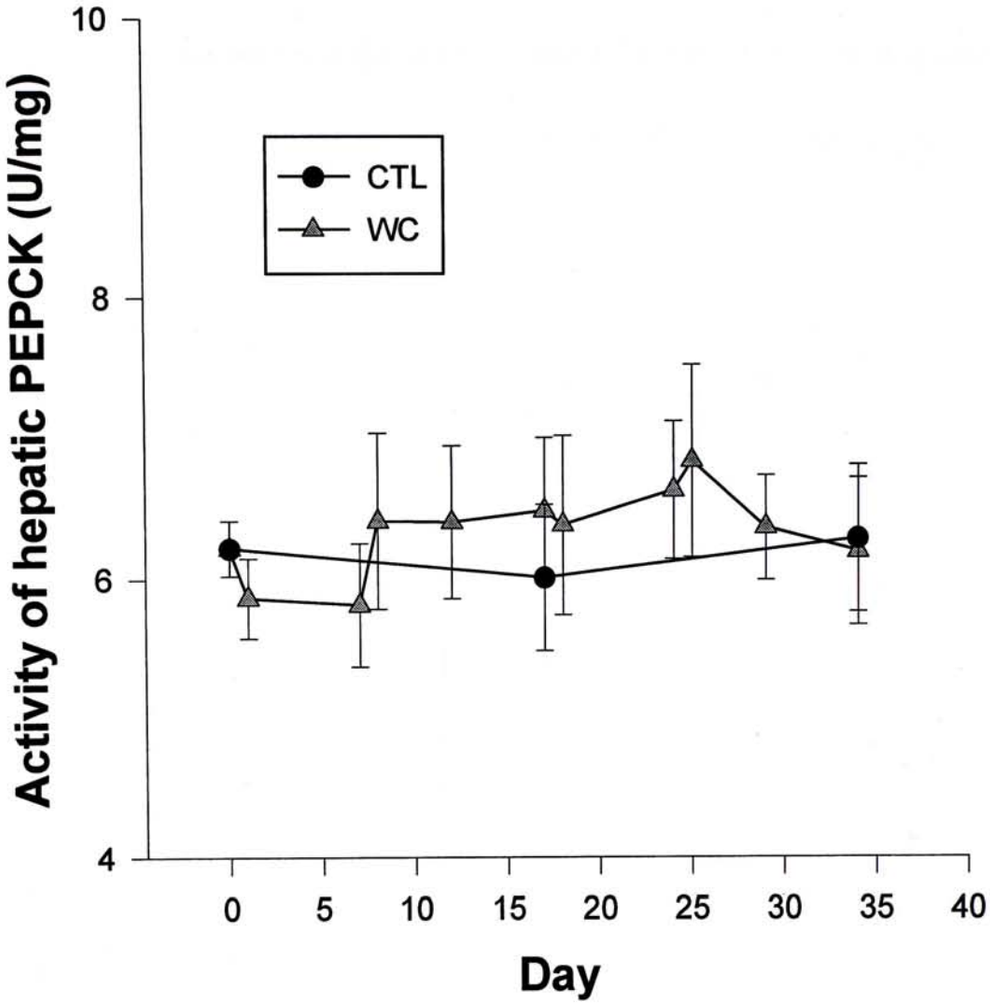


Figure 4.23 Specific activity of hepatic PEPCK of rats fed MF-diet.

4.4.2 Level of Serum Insulin and Glucagon

In both HF and MF groups, there was no significant difference in plasma insulin and glucagon between the WC rats and CTL rats during fasting and refeeding (Figures 4.24, 4.25, 4.26 and 4.27), though they fluctuated during the two weight cycles in WC rats.

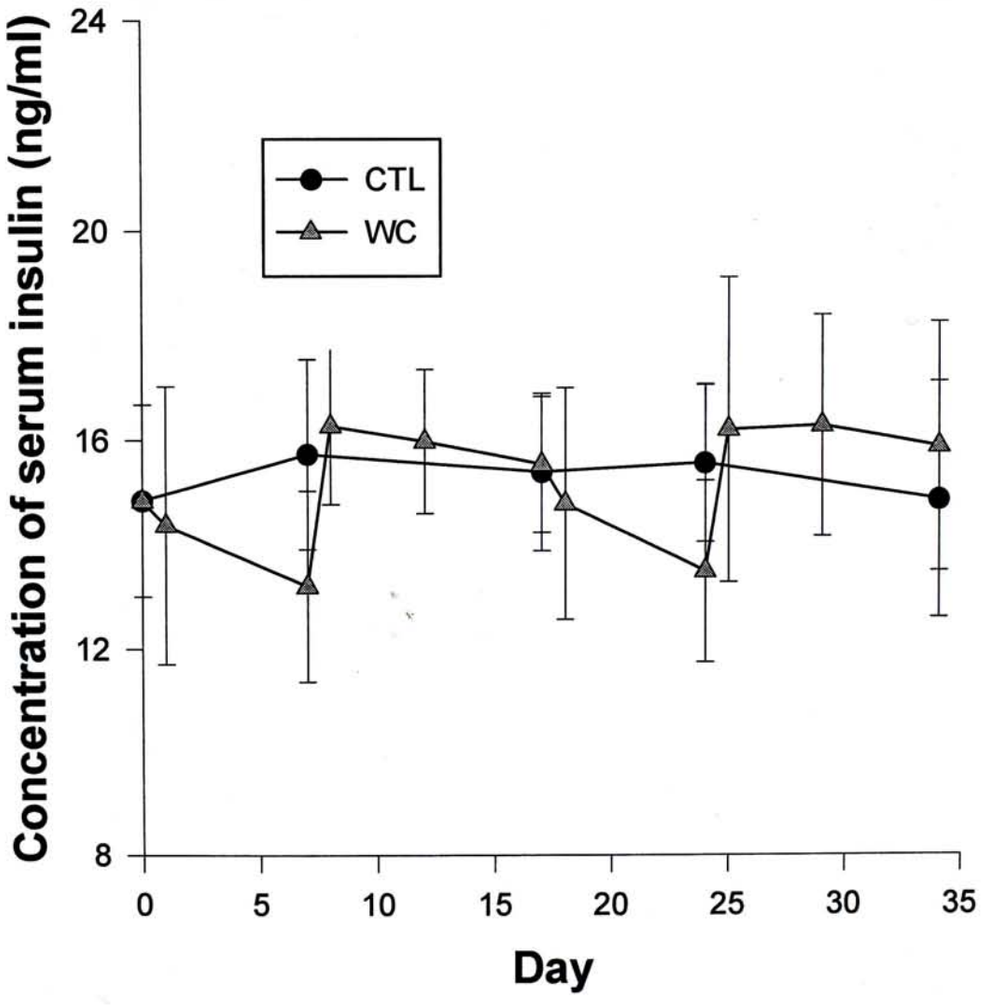


Figure 4.24 Effect of WC on serum insulin level of rats fed HF-diet.

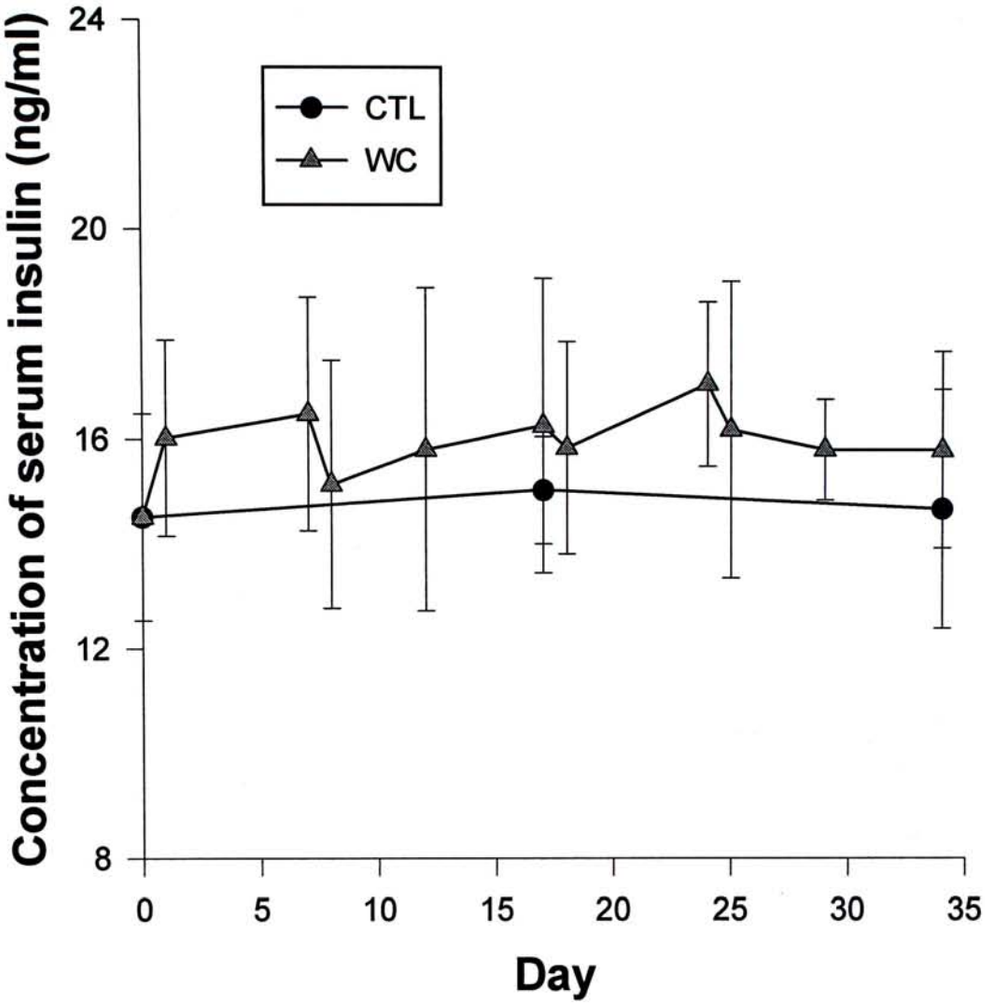


Figure 4.25 Effect of WC on serum insulin level of rats fed MF-diet.

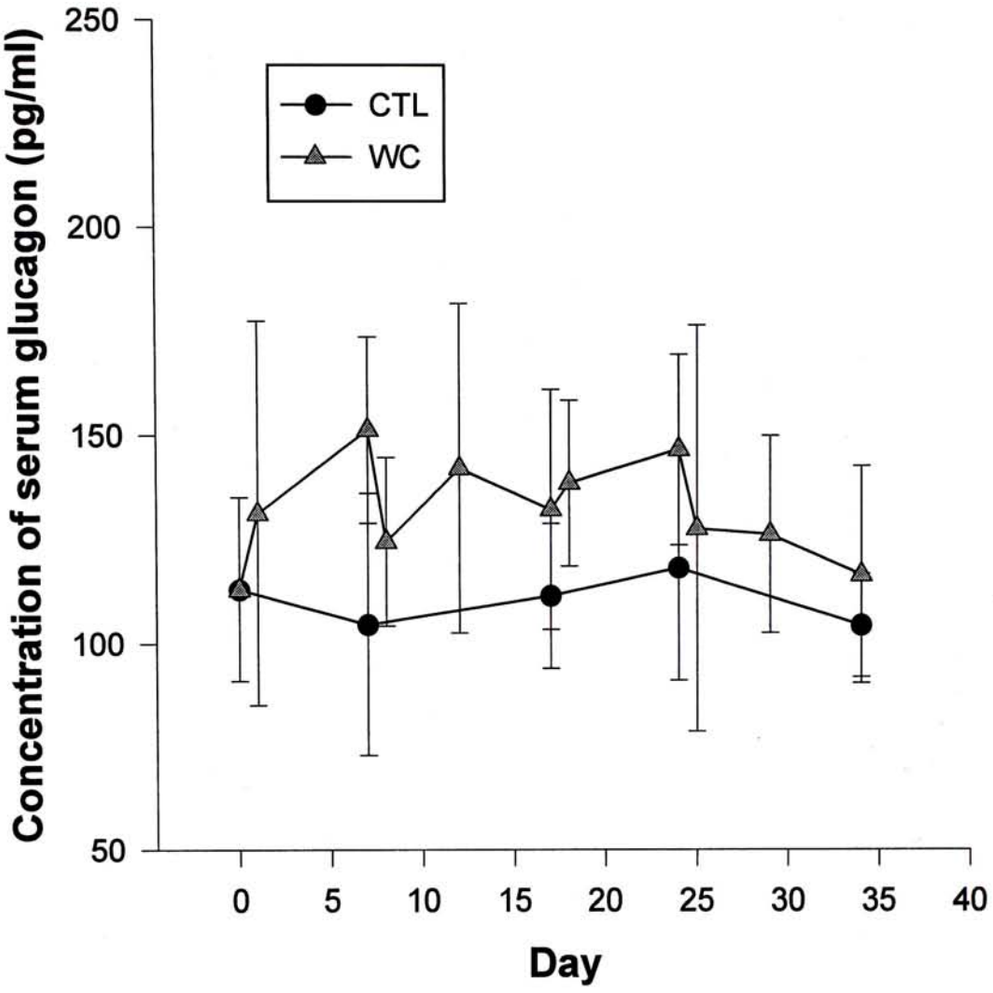


Figure 4.26 Effect of WC on serum glucagon level of rats fed HF-diet.

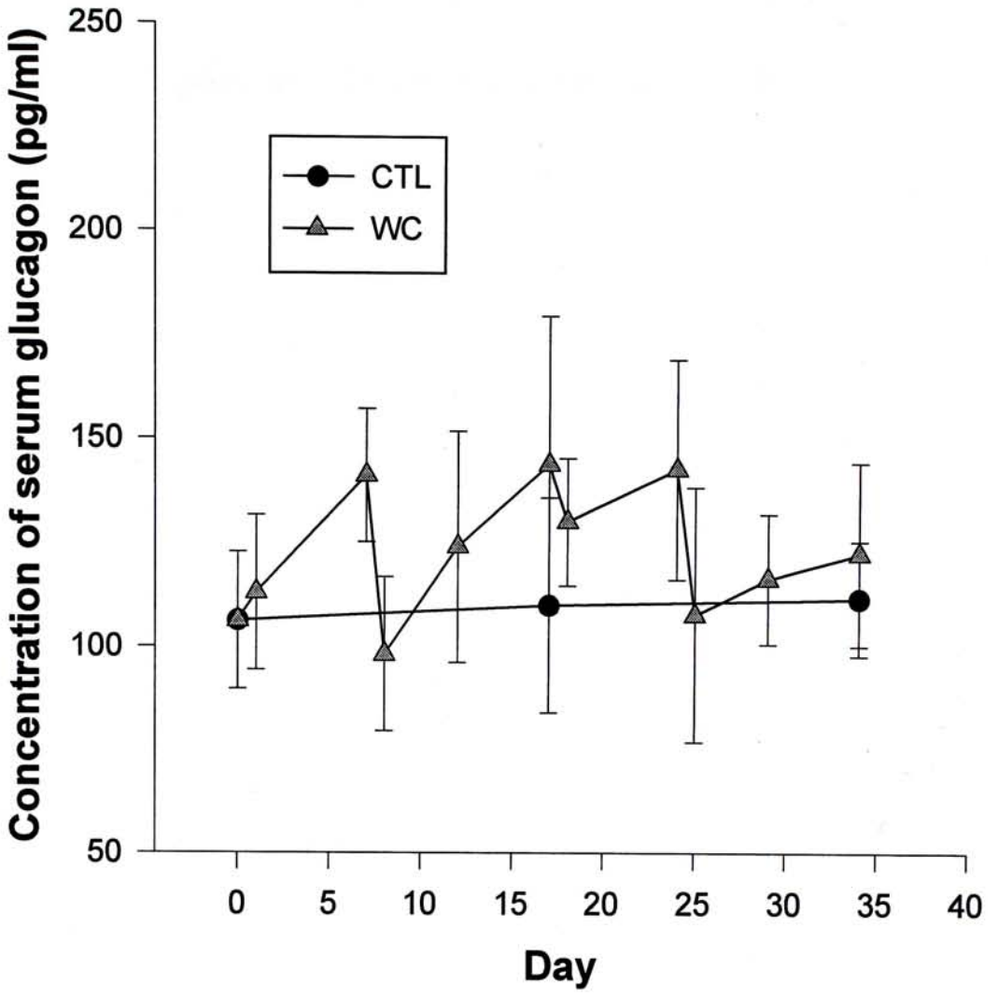


Figure 4.27 Effect of WC on serum glucagon level of rats fed MF-diet.

4.5 Discussion

4.5.1 Effect of Weight Cycling on Activity of Lipoprotein Lipase and Lipogenic Enzymes Activity

The present work clearly showed that LPL and lipogenic enzymes including FAS, ME and ACC were suppressed during the caloric restriction but there was a large induction and an overshoot of activity during refeeding regardless of the dietary fat level. This was consistent with previous studies (Fried *et al.,* 1983a; 1983b; Clearly, 1986a; 1986b; Lim *et al.,* 1996; Kochan *et al.,* 1997). However, the most interesting finding was that the response of WC rats fed HF diet was different from that of the WC rats fed MF diet. Firstly, the increase of lipogenic enzyme activities during refeeding was more pronounced in WC rats fed HF diet compared with the rats fed MF-diet. Secondly, the duration of the overshoot of the enzymatic activities was longer in WC rats fed HF diet compared with the WC rats fed MF diet. The WC rats fed MF diet showed an increase of the enzymatic activities in the early stage of refeeding periods and then approached gradually to the level of the control rats. Finally, at the end of the two cycles, the final enzymatic activity of WC rats fed HF diet was significantly higher than that of their CTL counterparts, whereas the WC rats fed MF diet had the final enzymatic activities close to their *ad libitum* fed CTL.

These discrepancies might confirm the previous findings that weight cycling induced obesity only when the rats were fed a high fat diet. In WC rats fed a HF diet, during the refeeding after the food restriction, lipogenesis increased considerably and maintained high throughout the 10 days of refeeding. Besides, the activity of LPL, a key regulator in fat accumulation in adipose tissue (Schotz *et al.,* 1990; Bensadoun,

1991), was also extensively increased during the whole refeeding periods. Therefore, once the WC rats was refed after the caloric restriction, the lipogenic enzymes were activated to cause hyperlipogenesis from the increased anabolism and the triglyceride endogenously synthesized or exogenously obtained from diet were then efficiently stored in the adipose tissue by the activated LPL. These two steps were stimulated continually until the next cycle started. As a result, the size of adipocytes were enlarged and gave heavier fat pads in WC rats (Figures 3.16, 3.10 and 3.11). In the WC rats fed MF diet, the smaller degree and shorter duration of the increase of enzymatic activities might only allow the rats to replenish their previous fat cell size and weight of fat pads.

Another interesting finding in this study was that the overshoot of the activities of FAS, ME and PK of WC rats in the second refeeding was more considerable than that in the first refeeding. This is consistent with the study of Kochan *et al.* (1997) who showed that several WCs induced higher activities of the lipogenic enzymes.

ACC is the key enzyme catalyzing the rate-limiting step in the fatty acid synthesis. Although its activity also gave an overshoot in the refeeding periods, the extent was much smaller than the other lipogenic enzymes. The reason for this was unclear but it might be partly explained by the fact that high ionic strength and gel filtration used in preparing the crude enzyme might cause some lose of ACC activity (Salati and Goodridge, 1996). Another lipogenic enzyme, PEPCK, only gave a small response during the fasting-refeeding cycles. This may be due to the role of this enzyme is less crucial in the fatty acid synthesis compared with other lipogenic

enzymes. Therefore, its sensitivity to the change of the physiological state was relatively lower.

4.5.2 The Overshoot of Enzymatic Activities in Relation to Tissue Fatty Acid Composition

The previous studies (Chapters 2 and 3) showed that weight cycling caused an increase of SFAs with concomitant decrease of PUFAs in adipose tissue and carcass. It was also proposed that the subsequent stimulated lipogenesis during refeeding following fasting increased the amount of SFAs, which in turn, dilutes the proportion of PUFAs. This was well confirmed by the present results. As only SFAs can be *de novo* synthesized, the amount of SFAs is hence increased considerably during refeeding. The increase in the activities of the lipogenic enzymes would increase the fatty acids synthesis. Furthermore, the lipid in adipose tissue is mainly triglycerides. Therefore, the increased LPL activity during refeeding resulted in an efficient storage of the newly synthesized SFAs into adipose tissue which thus showed a higher SFAs profile. However, the reason for alteration of fatty acid profile only in carcass TG but not carcass PL in WC is still poorly understood. Our present results cannot explain this observation as the synthesis and hydrolysis of TG and PL undergo different pathways. Hence, further investigations linking the effect of weight cycling on TG and PL metabolism are needed.

4.5.3 No Elevation of Plasma Insulin in Weight Cycled Rats

It has been discussed in Section 4.1.2 that the fatty acid synthesis and storage are stringently regulated and it is achieved by the actions of insulin and glucagon on the enzymatic activities of LPL, ACC, FAS, ME and PK. These enzymes showed a decrease of activity during fasting and an increase in refeeding. Insulin also enhances the enlargement of adipocytes and energy storage (Bjorntorp, *et al.,* 1980; Cushman, *et al.,* 1981) and hyperinsulinemia has been reported in weight-cycled rats (Contreras and Valerie, 1989). It was thus supposed that a reduction of insulin-glucagon ratio in fasting and an elevation of the ratio in refeeding would occur. Though there was slight fluctuation of the levels of these two hormones during the two weight cycles, the concentration of plasma insulin and glucagon in the WC rats was not statistically significantly different from that in the CTL rats at the end of two weight cycles. Therefore, the mechanisms by which weight cycling increases activity of lipogenic enzymes and LPL remained poorly understood.

Chapter 5 Conclusion

In recent years, weight cycling or "yo-yo" dieting has been used in the fields of nutrition and obesity research, and it refers to repeated losses and subsequent regains of body weight. Weight cycling may pose some negative impact on health (Ernberger and Nelson, 1988; Robert and Williams, 1989; Wannamettee and Sharper, 1990; Ernsberger *et al.,* 1995). However, it remains controversial whether weight cycling is associated with higher risk of CHD (Lissner and Brownell, 1992). Furthermore, the statement "the potential hazards of weight cycling are outweighed by the benefits of possibly losing excess body fatness" is often debated among the health professionals (Anonymous, 1994). The inconsistency of the findings may be due to the various criteria used to define weight cycling.

In spite of the controversy, the present study did demonstrate that weight cycling disturbs the fatty acid balance in the adipose tissue and carcass when rats are used as an animal model. Firstly, this alteration with an increase of SFAs and a preferential reduction of LA and α-LnA is independent of the extent of caloric restriction and the level of dietary fat. Secondly, weight cycling with a high-fat diet caused an enlargement of adipocytes and an increase of adipose fat pad weight. Finally, the changes in fatty acid composition and the size of adipose tissue were parallel to an overshoot in activity of LPL and lipogenic enzymes. This overshoot of activity was more pronounced in weight cycled animals fed a HF diet and probably caused hyperlipogenesis during the refeeding. Since large amount of SFAs were synthesized during refeeding, eventually it resulted in a higher proportion of SFAs but

a relative lower proportion of LA and α-LnA after the weight cycles. Moreover, the newly synthesized fatty acids were stored up efficiently in adipose tissue by activated LPL and thereby, causing larger adipocytes and heavier fat pads.

Although the present results cannot be directly transferred to human conditions, it may have implication for individuals undergoing the inevitable weight regain after prolonged hypocaloric treatment of obesity. Likewise, the results suggest that it may not be healthy for people who are not overweight but dieting frequently only for the sake of keeping body image.

Overweight or obesity is a serious nutritional problem in the developed countries. Though billion of dollars was spent on investigating the best way to lose the excessive body fat every year, dieting is still commonly practiced and may eventually develop a "yo-yo" pattern. On the basis of existing researches, there is no reason to discourage overweight patients from losing weight but weight cycling should be avoided.

Insulin and glucagon play crucial roles in regulating lipid. It is known that insulin controls the activities of lipogenic enzymes and LPL by altering their gene expressions (Goodridge, 1987; Bensadoun, 1991; Salati and Goodridge, 1996). The recent report by Contreras and Valerie (1989) has shown that weight cycling causes hyperinsulinemia in rats. However, we failed to demonstrate that there is a significant difference in the level of serum insulin and glucagon between the weight cycled and control rats. The underlying mechanisms for the elevated activity of lipogenic enzymes in the weight cycled animals fed a high-fat diet remains poorly understood.

Perhaps, weight cycling may affect the gene transcription or translation and expressions of these enzymes. Moreover, it will be very interesting to address how long the overshoot of the enzymatic activity lasts during *ad libitum* refeeding. It will also be curious to know how much 18:2n-6 and 18:3n-3 can drop in the adipose tissue and carcass if the number of consecutive weight cycles is increased. All these questions are presently unknown and deserve further investigations. The impact of weight cycling on health remains mysterious not only to nutritionists, but also to biochemists and molecular biologists.

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